The biogeochemistry of Pu mobilization and retention

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Historically, expectations of minimal aqueous Pu transport of have been confounded by the apparent contradiction:

• an understanding of low Pu solubility (based on its inorganic speciation) and

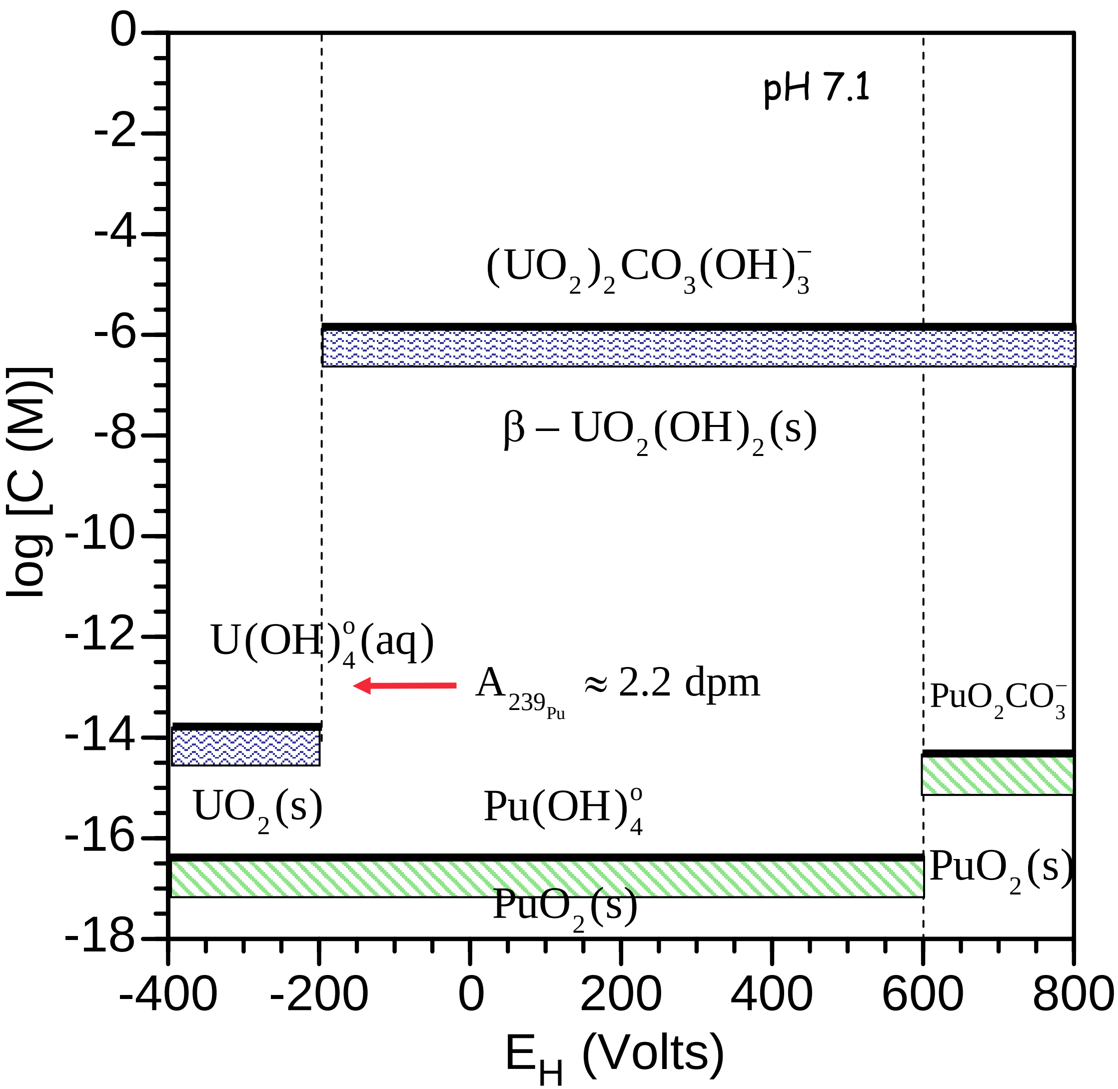
• the observed transport of Pu sometimes at substantial distances from its presumed source.

Of the relatively limited number of papers on Pu environmental speciation, a majority have implicated 'organic matter' as a transport agent*.

This project focuses on the role of bacteria in the production of EPS: as Pu mobilization and immobilization agents.

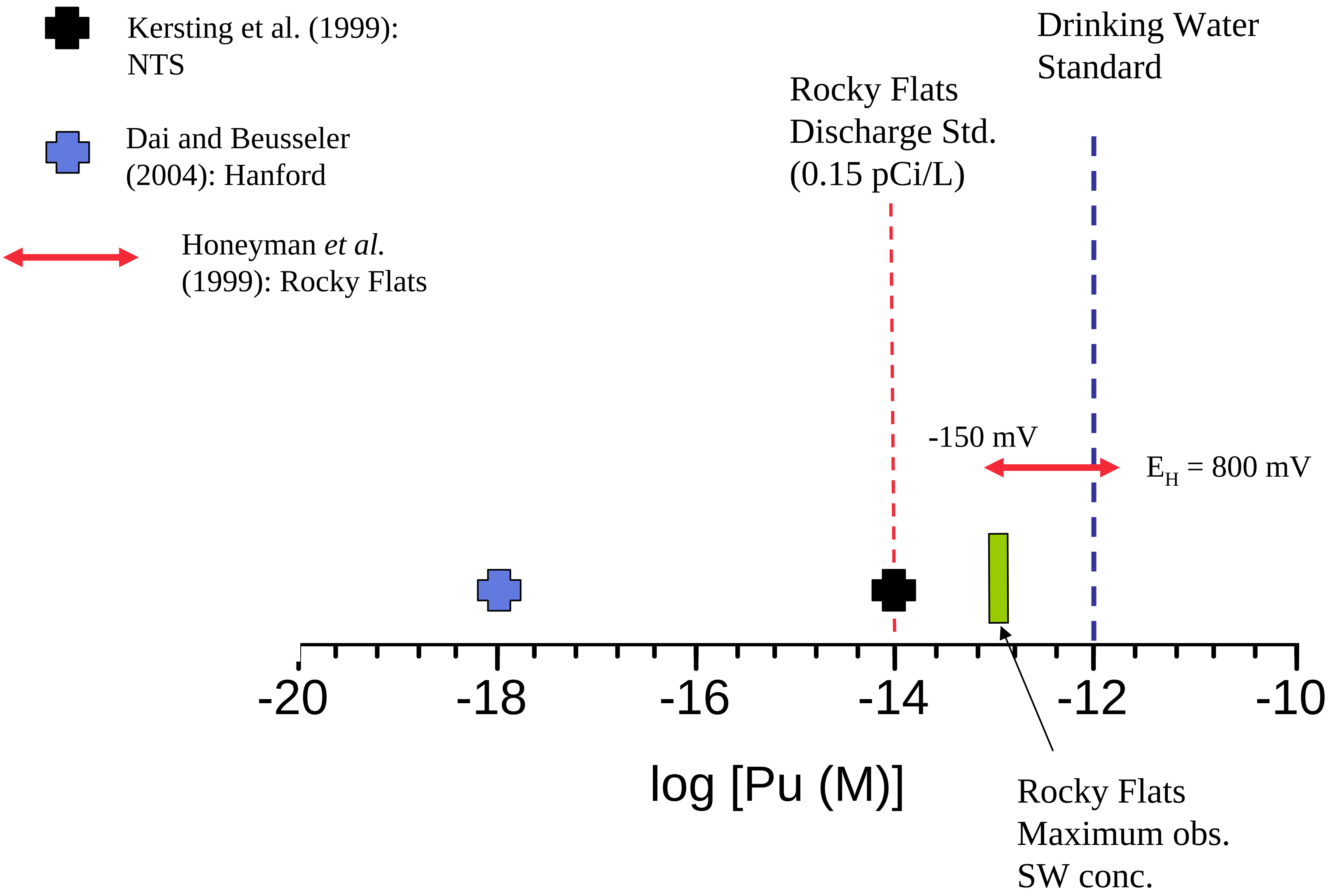
*(e.g., Nelson *et al.,* 1990; Orlandini *et al.,* 1990; Loyland *et al.,* 2001; Honeyman, 1998; Santschi *et al.,* 2002)

Comparison of U and Pu solubility

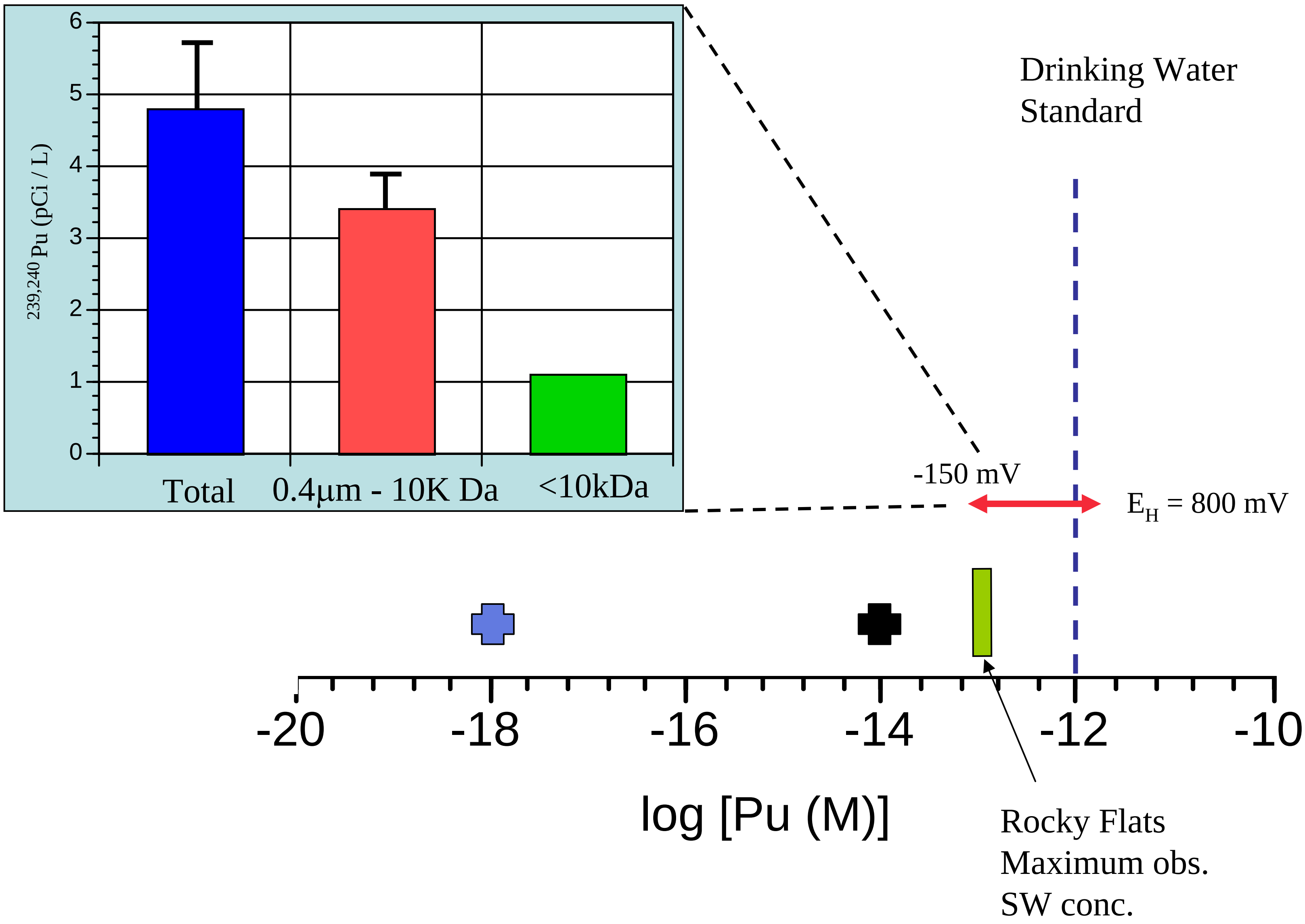


Uncertainty over controlling phase

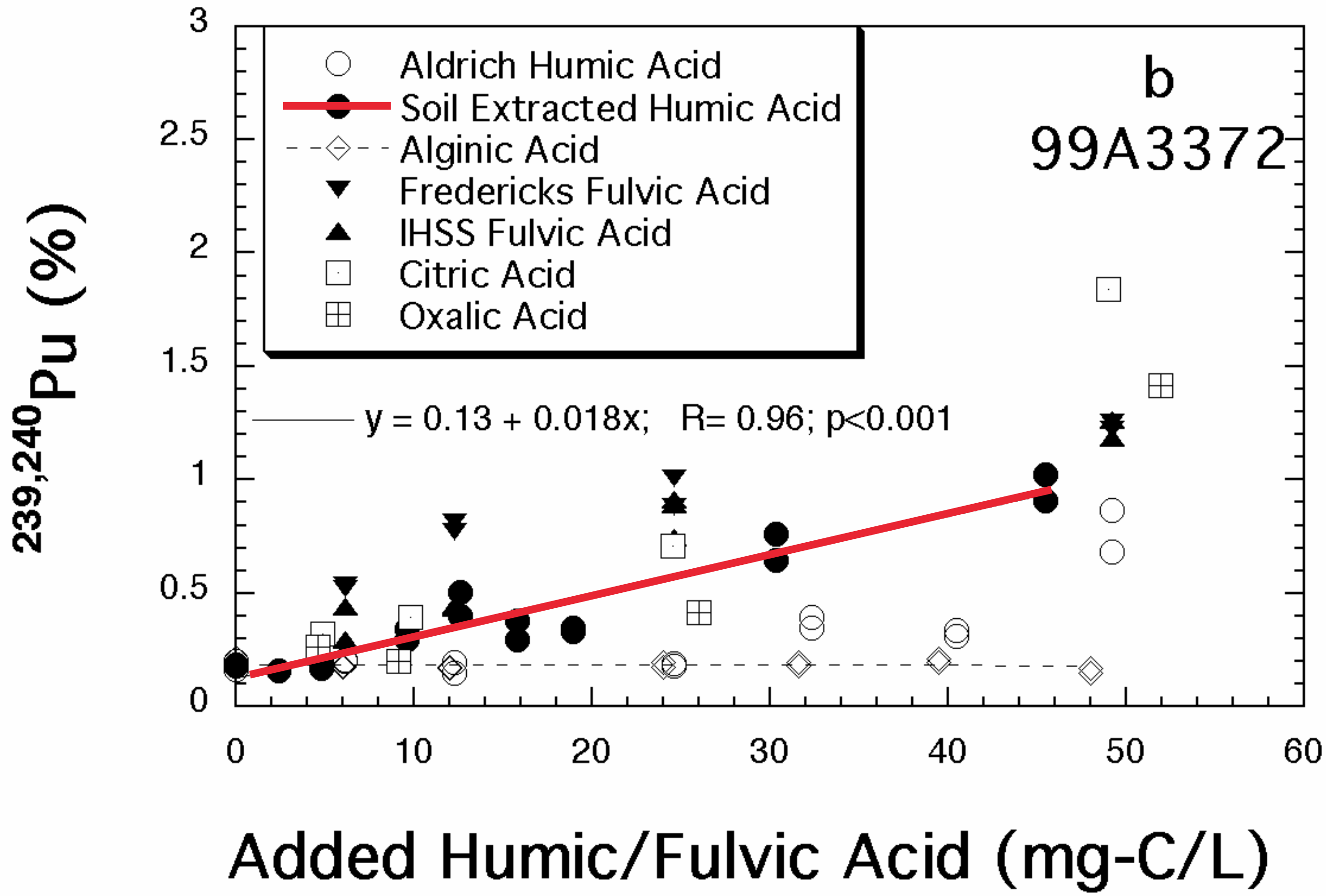
Examples of environmental measurements



Examples of environmental measurements

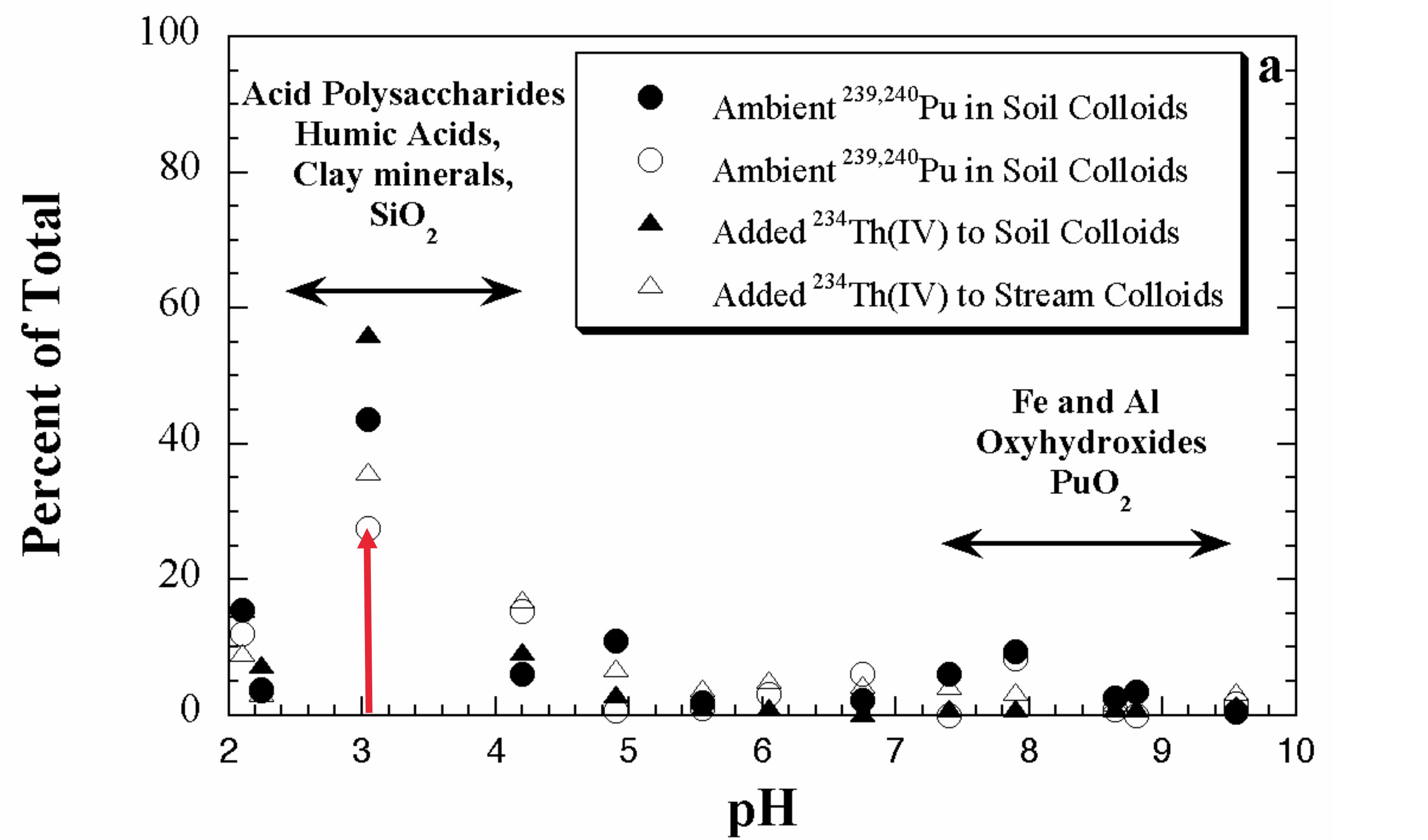


Pu release from vegetated soils as a function of quality and quantity of DOC



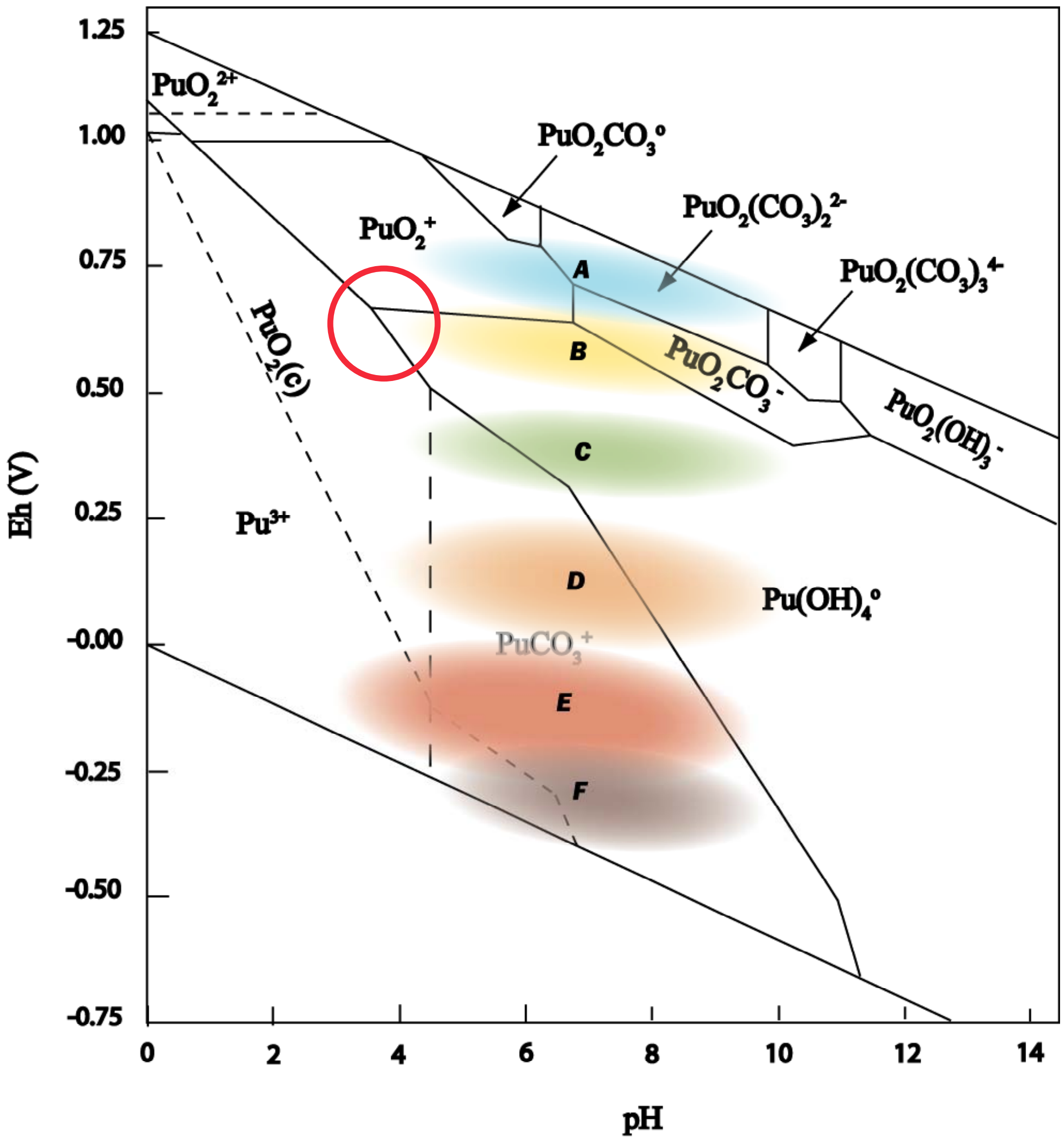
Santschi *et al.* (2002) Environ. Sci. Technol., 36, 3711-3719.

PAGE (Gel electrophoresis) of $^{239,240}Pu$ from Soil Resuspension Experiment



Santschi *et al.* (2002) Environ. Sci. Technol., 36, 3711-3719.

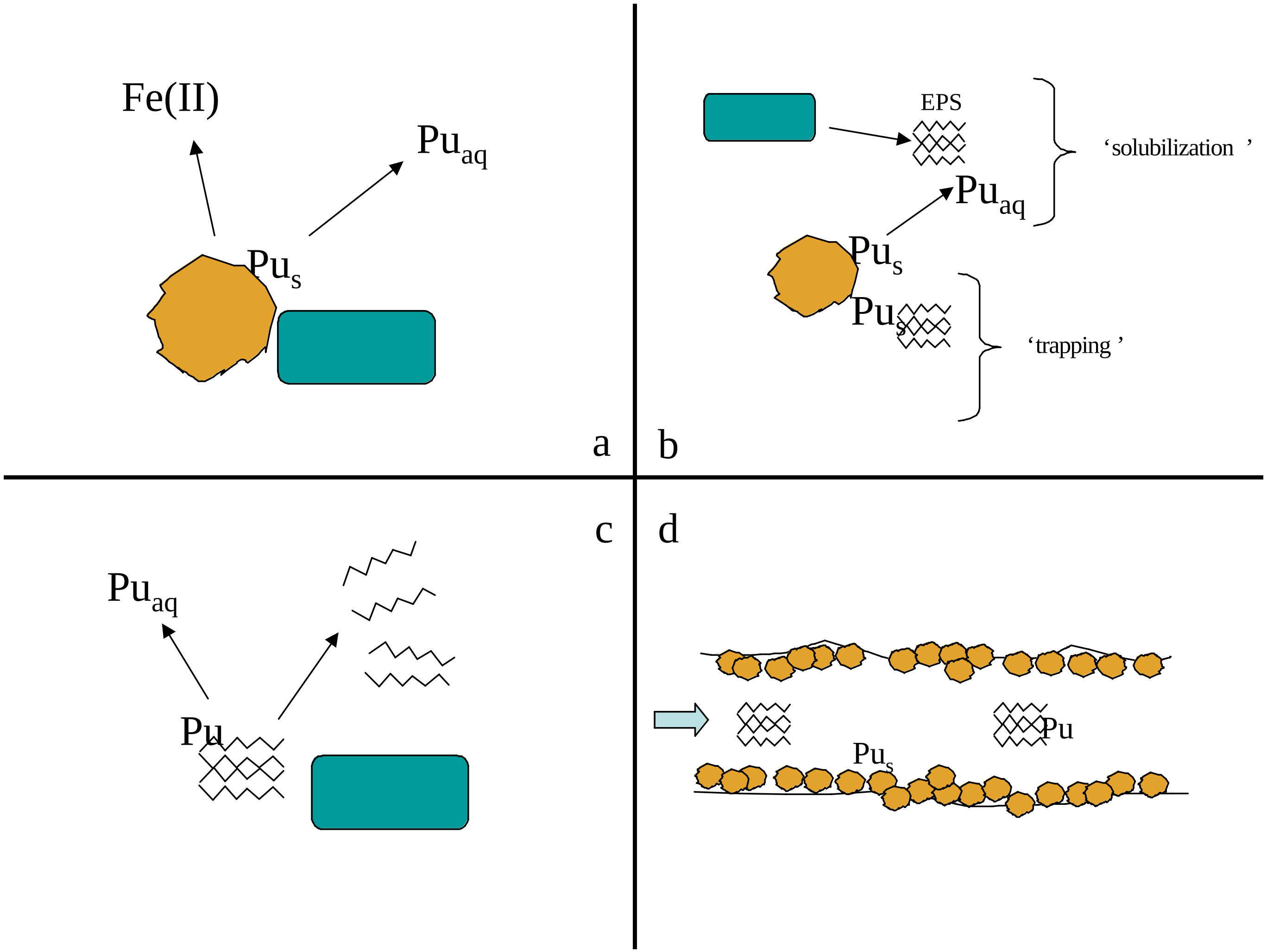
Pourbaix Diagram (Eh/pH) diagram for the system Pu-O_2-CO_2-H_2O at 25 °C and 1 bar total pressure for Σ Pu(aq)=10^{-8} M and total carbonate (C_T) = $10^{-2.0}$ M

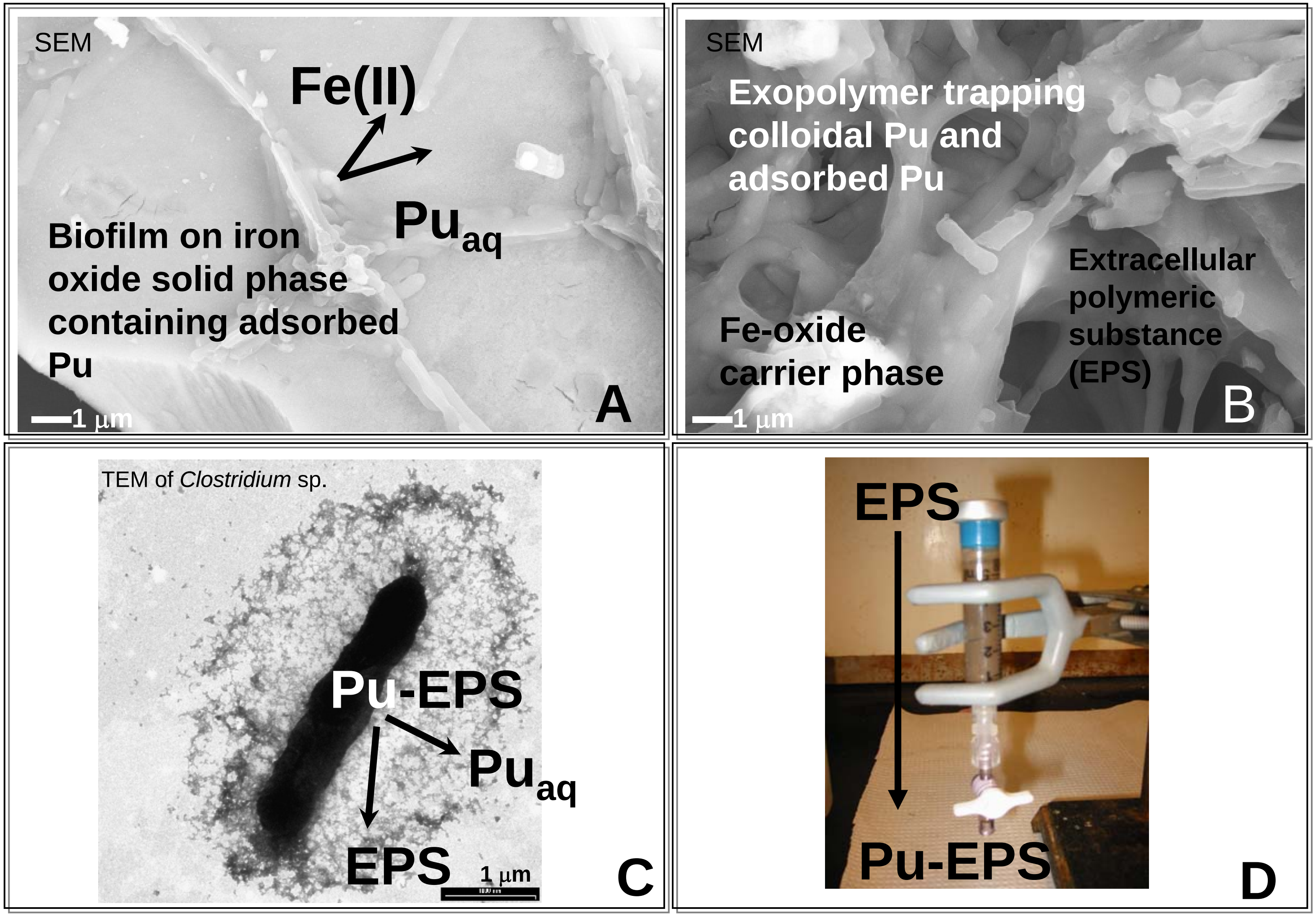


A: Aerobic bacteria
B: Denitrifers
C: Mn-reducers
D. Fe reducers
E: Fermenters
F: Sulfate reducers

After Langmuir (1997)

Pu mobility / immobility as a transformational process

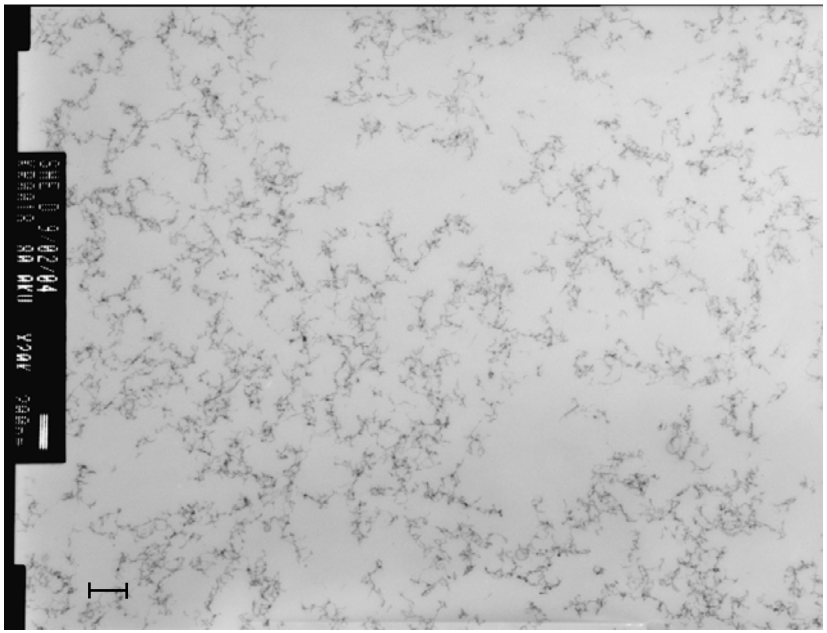




Key: **A**. Pu carrier-phase dissolution; **B**. Trapping of colloidal Pu by EPS; **C**. Biodegradation of Pu-EPS; **D**. Enhanced Transport of Pu by EPS.

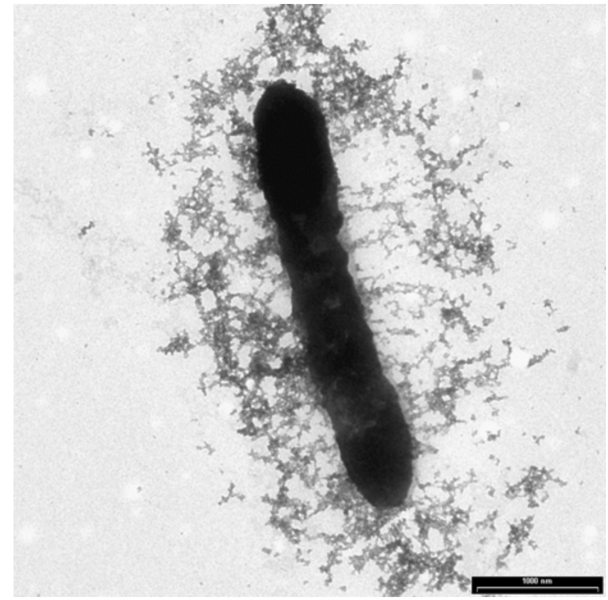
Characterization of EPS

TEM of <0.45 μm EPS from Shewanella putrefaciens



Bar = 200 nm

TEM image of Clostridium sp.



J.B.Gillow (unpublished)

TEM image of *Clostridium* sp. after 48 hours growth showing polysaccharide surrounding cell (bar = 1000nm)

Soil Bacterial cultures

(C) Centrifugation (3600 g, 30 min)

Pellet

Dissolved in D.W. with 3% NaCl for 1 hr (C)

Capsular exopolymer

Supernatant

(C) EtOH precipitation (overnight, filtration)

Pellet

(C) Dissolved in D.W. with 3% NaCl, stirring 1 hr, add EtOH

Crude exopolymer

(C) Purification of EPS for three times with EtOH,

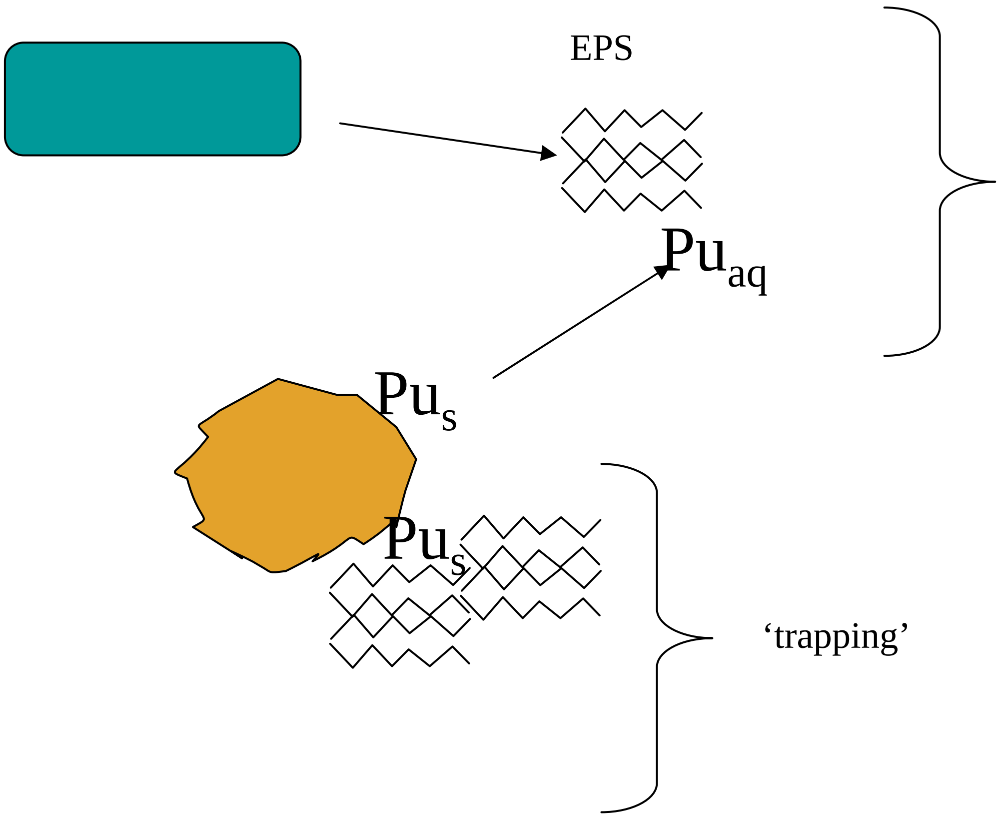
Mixture

(C) Removing Protein with proteinase or pronase for 12 hr at 37 C, then dialyzed, lyophilized

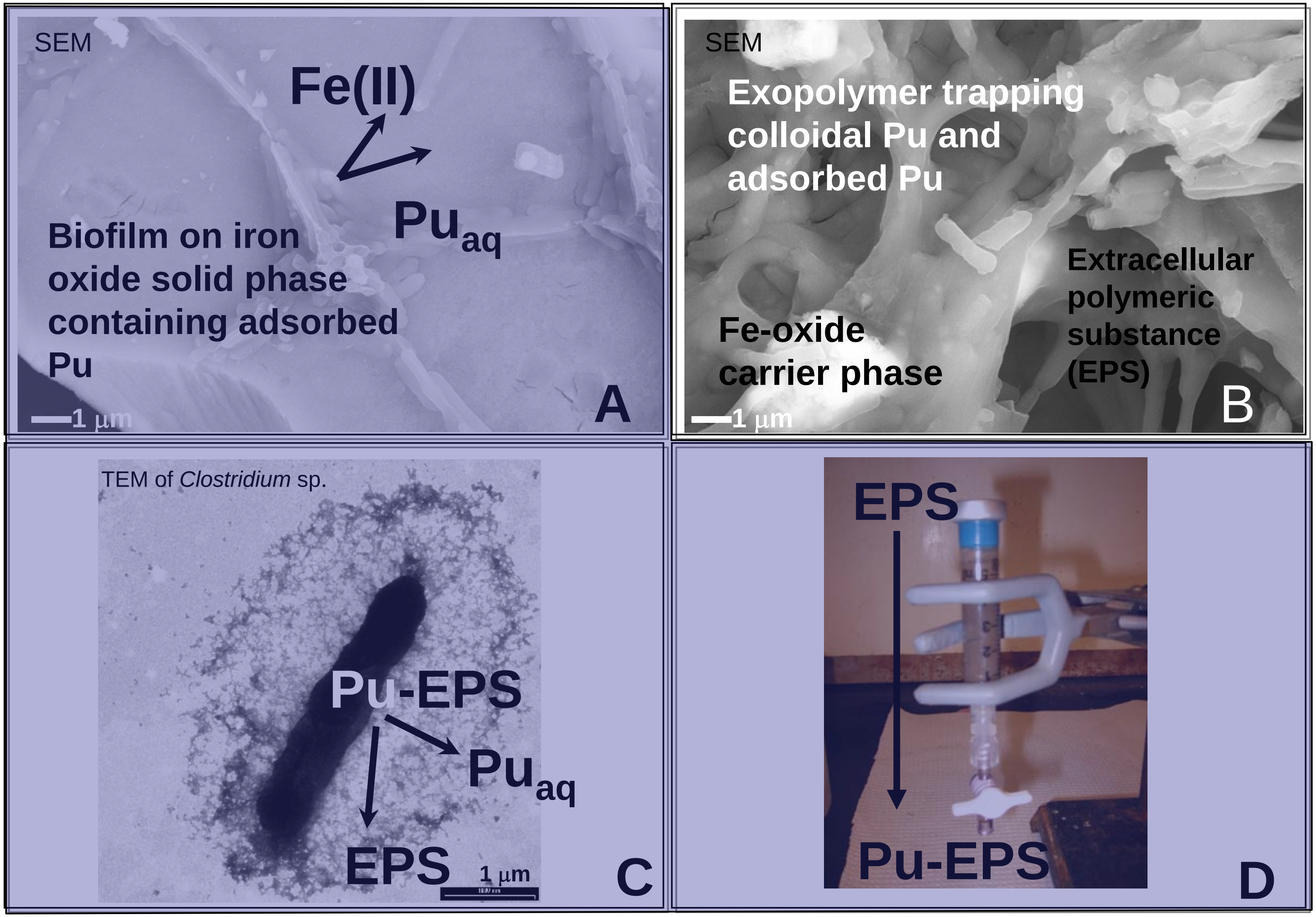
Pure EPS

Objectives

- Determination of amphiphilic character of EPS through evaluation of chemical composition of hydrophilic carbohydrates and uronic acids, and more hydrophobic proteins, through the use of Hydrophobic Interaction Chromatography (HIC).
- Pu(IV,V) partitioning to EPS from Pseudomonas with(w/) and without(w/o) proteins.



EPS
Pu$_{aq}$
Pu$_s$
Pu$_s$
'trapping'



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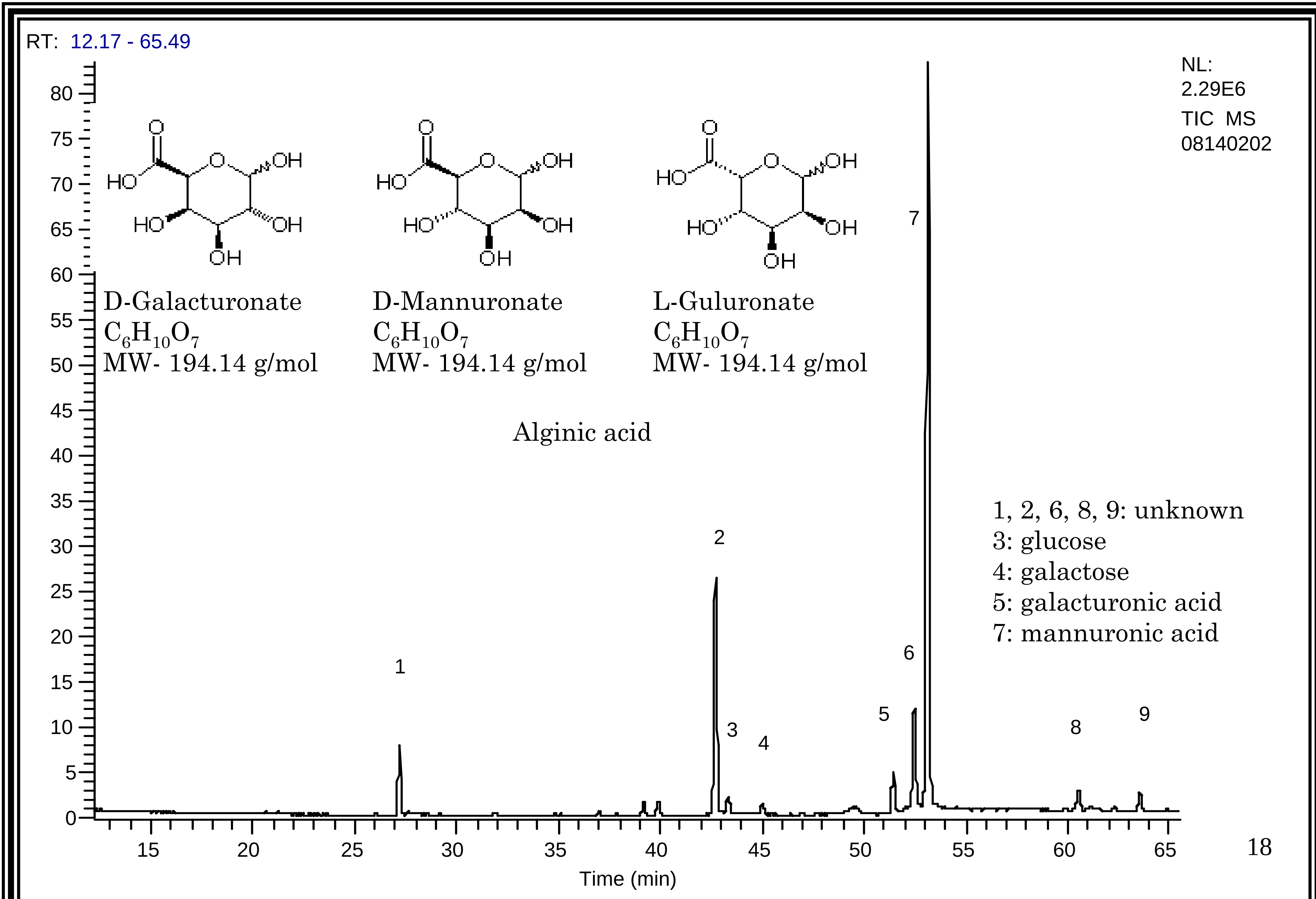
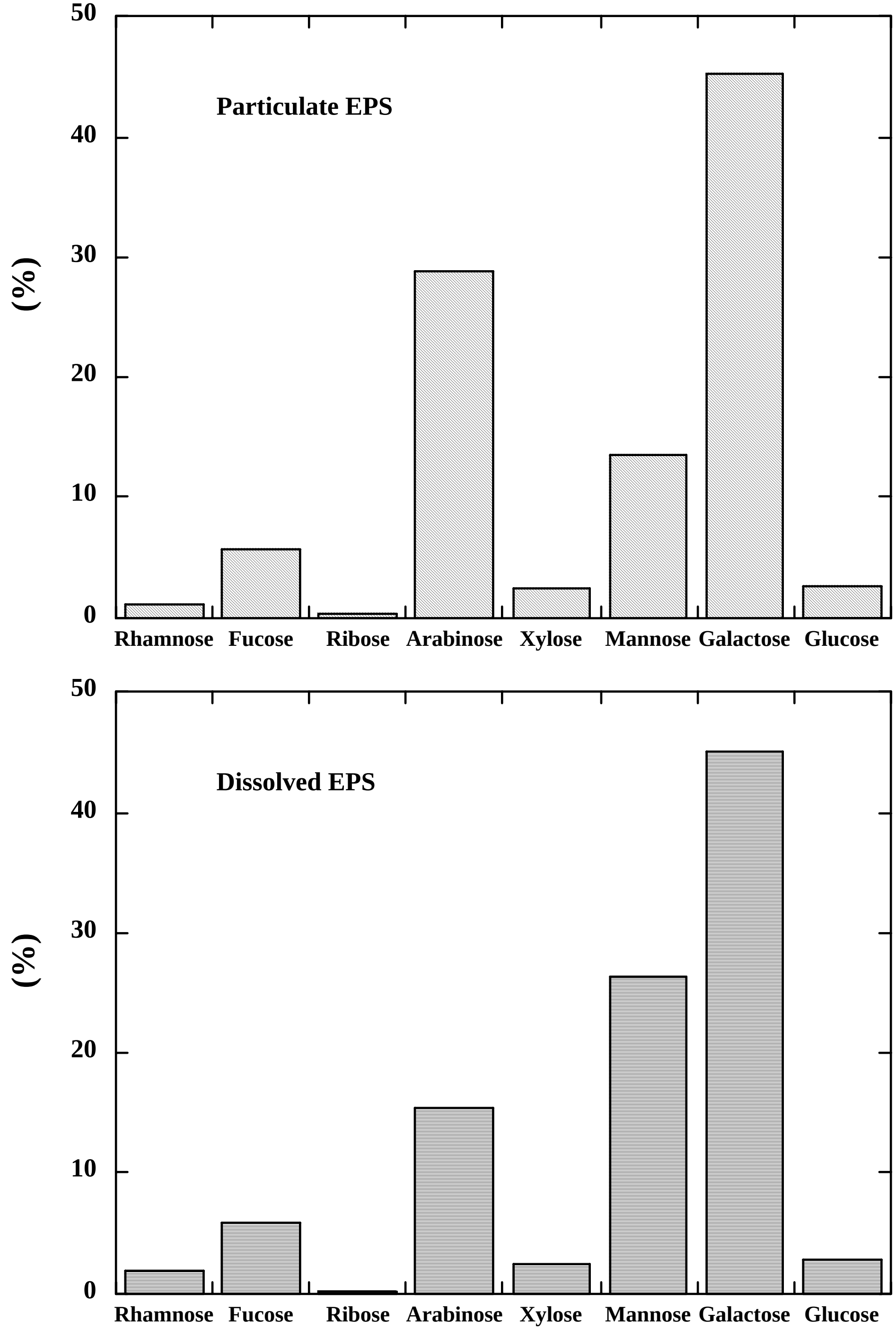
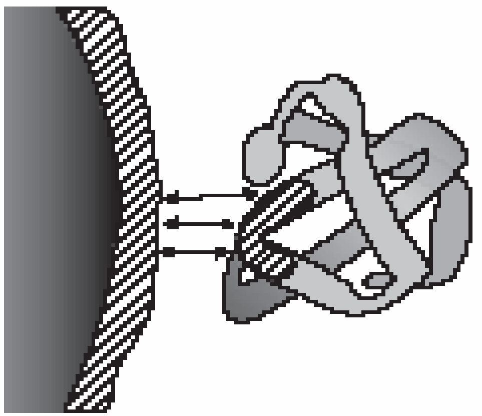
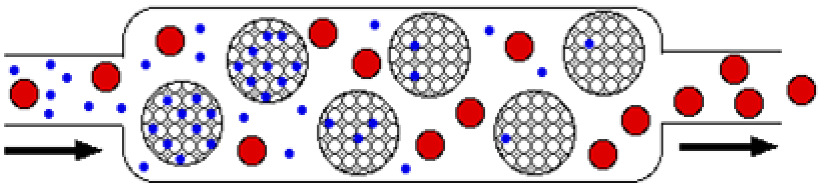
GC-MS Spectra of EPS (C.-C. Hung): *Clostridium sp.* Exudates (> 6 kDa)




Particulate EPS
(%)
50
40
30
20
10
0
Rhamnose
Fucose
Ribose
Arabinose
Xylose
Mannose
Galactose
Glucose
Dissolved EPS
(%)
50
40
30
20
10
0
Rhamnose
Fucose
Ribose
Arabinose
Xylose
Mannose
Galactose
Glucose

Hydrophobicity and MW determinations:



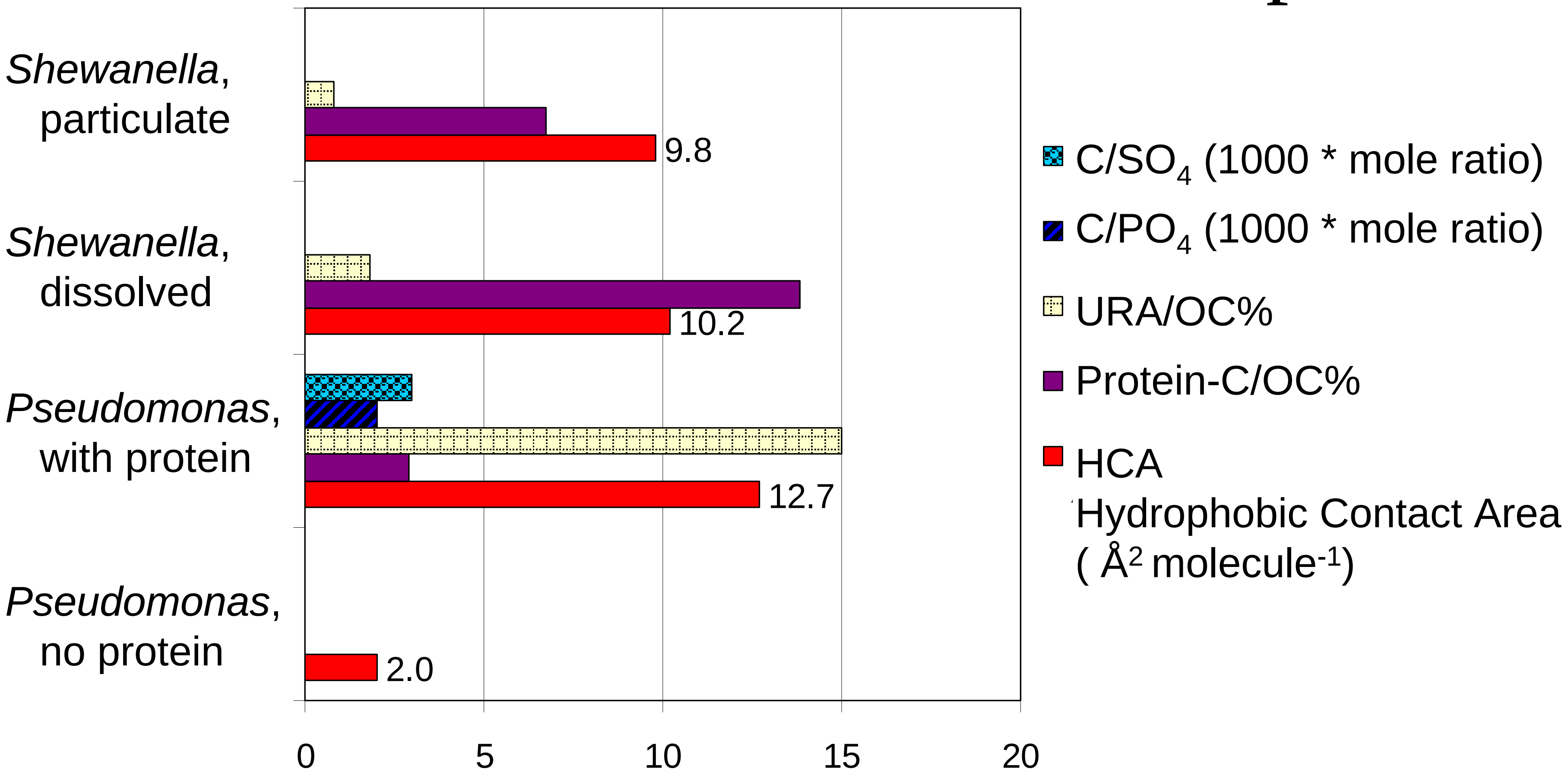


- Waters HPLC
- Amersham HiTrap, 2 butyl

1 ml columns in series

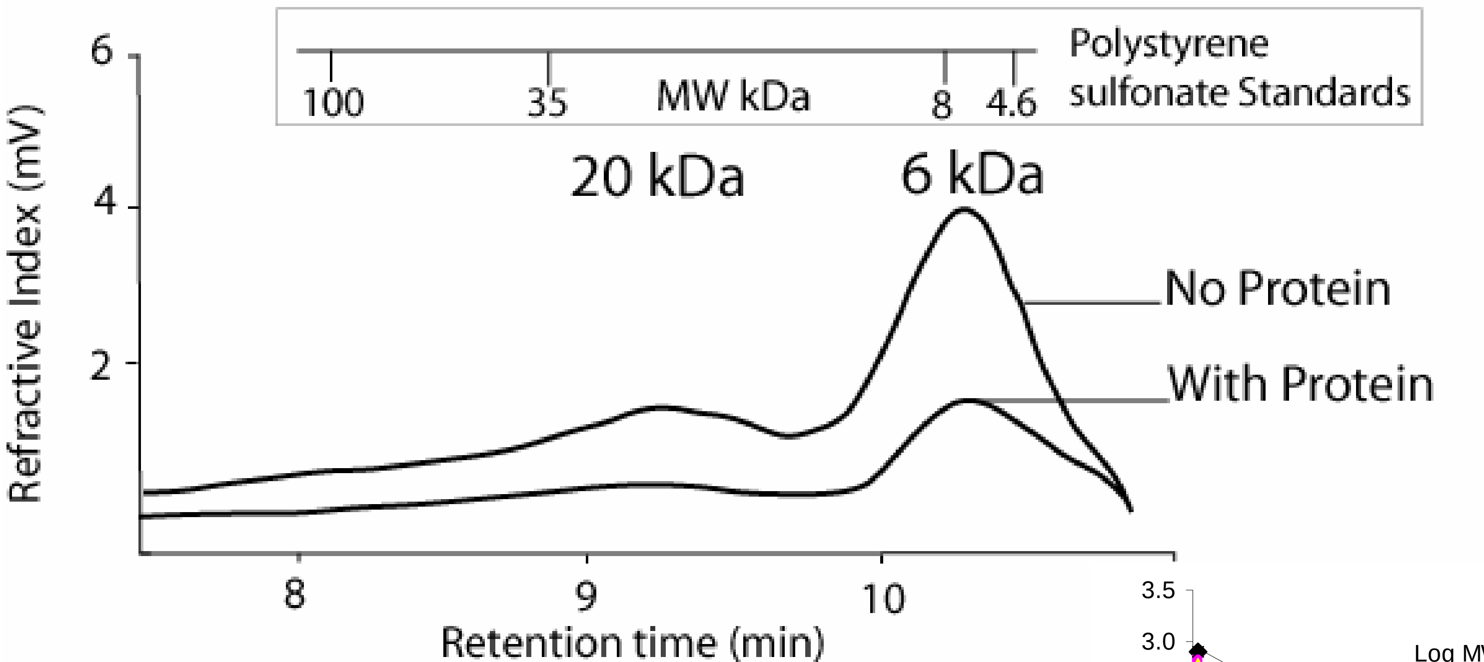
- Waters HPLC
- Tosoh Biosciences G4000 PWxl, 7.8 mm x 30 cm, particle size 10 μm, with guard column 6 mm x 4 cm

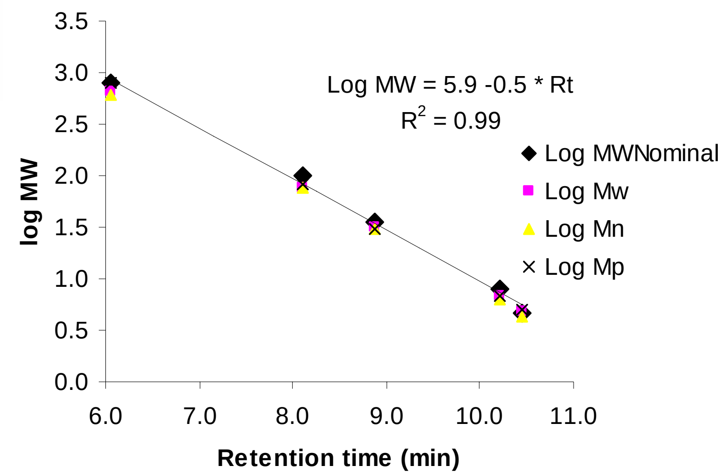
Results
HCA, Protein, Charged Groups



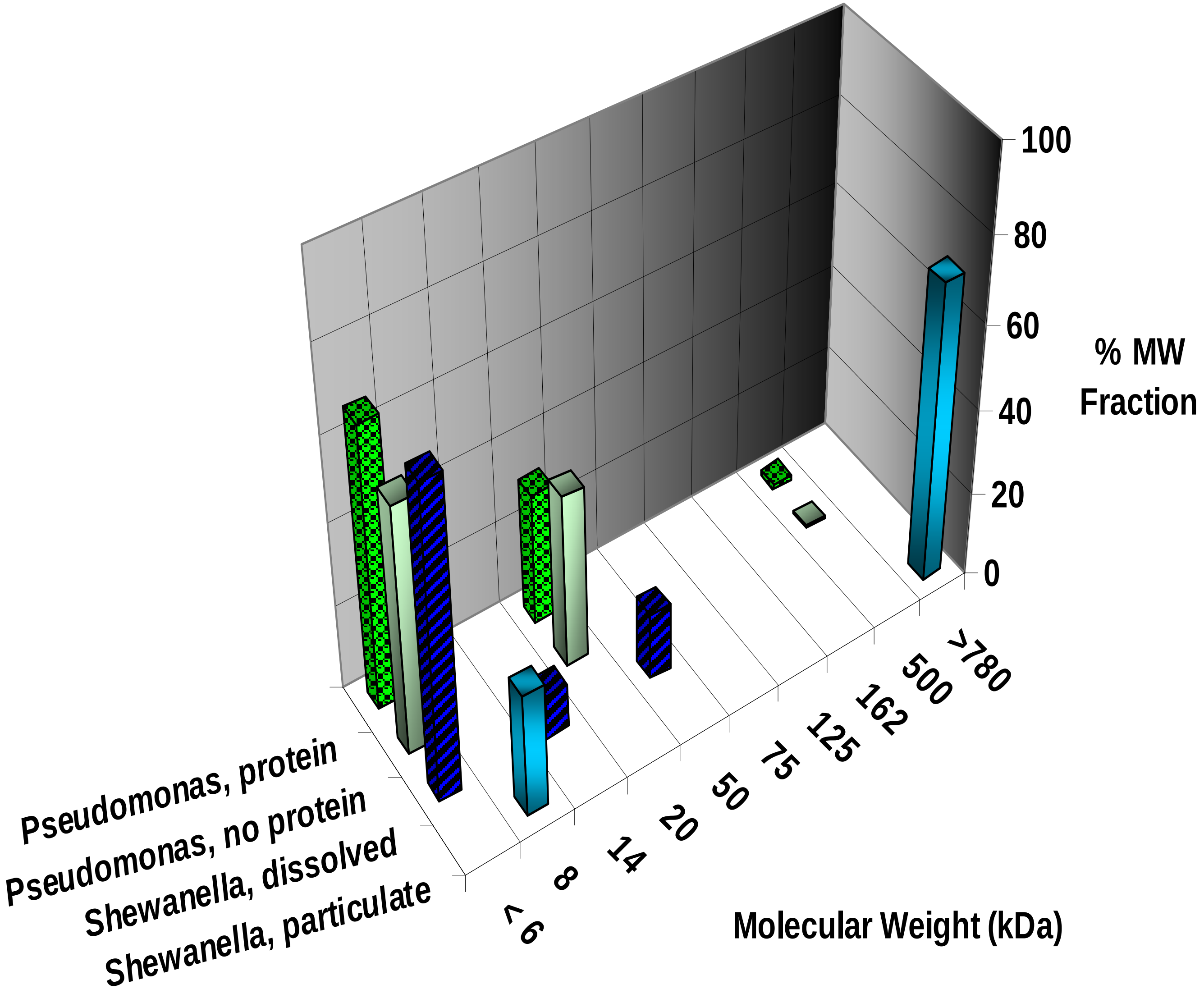
Results
Molecular Weight (MW) by SEC

Overlain SEC Chromatograms of Pseudomonas florescens



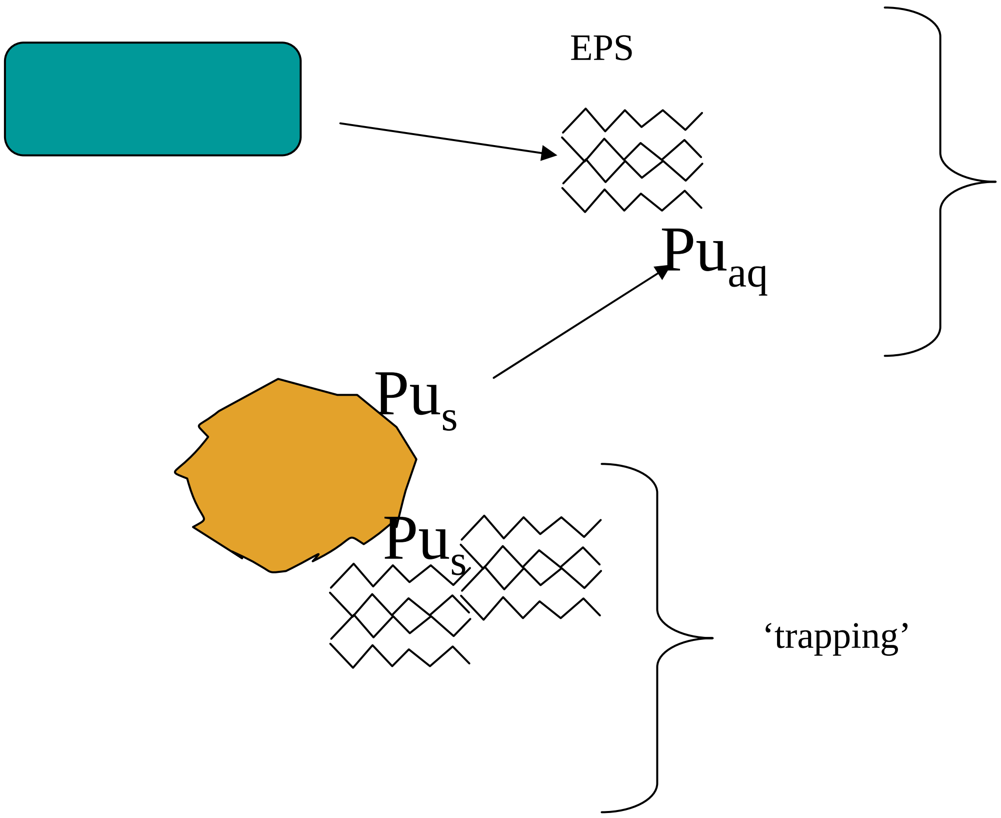


Results
Molecular Weight (MW) by SEC



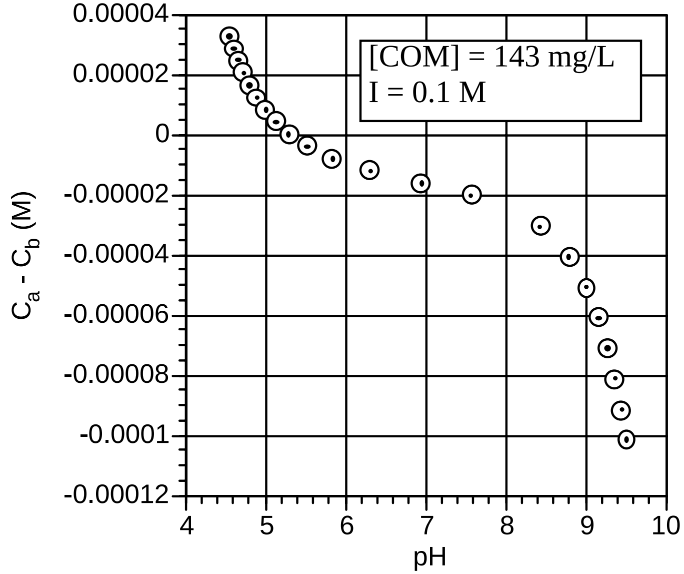
Summary of characterization work:

- The neutral monosaccharides in this EPS consist of rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose and glucose.
- The acidic groups in this EPS are mainly composed of carboxylic acid and minor polyanionic groups, e.g., sulphate and phosphate.
- 45 - 70 % of total carbohydrates are uronic acids, and total carbohydrates made up 26-31% of organic carbon.
- Besides the neutral and acidic sugars in the EPS, EPS also contained 9 % of proteins (% of carbon), which makes the EPS amphiphatic (amphiphilic).


EPS
Pu
aq
Pu
s
Pu
s
'trapping'

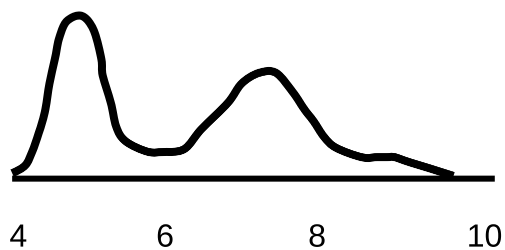
Pu / EPS complexes

- Evaluation of Pu binding sites (‘empirical adequacy’): affinity distribution
- Determination of conditional Pu /EPS complexes: ligand competition method



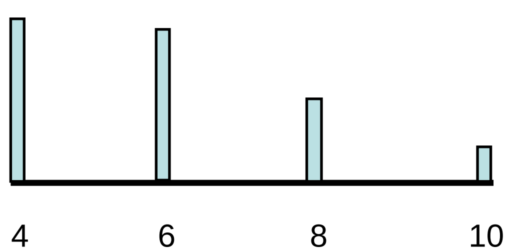
Linear combination of independent ionizable sites?

'Smoothness Hypothesis'

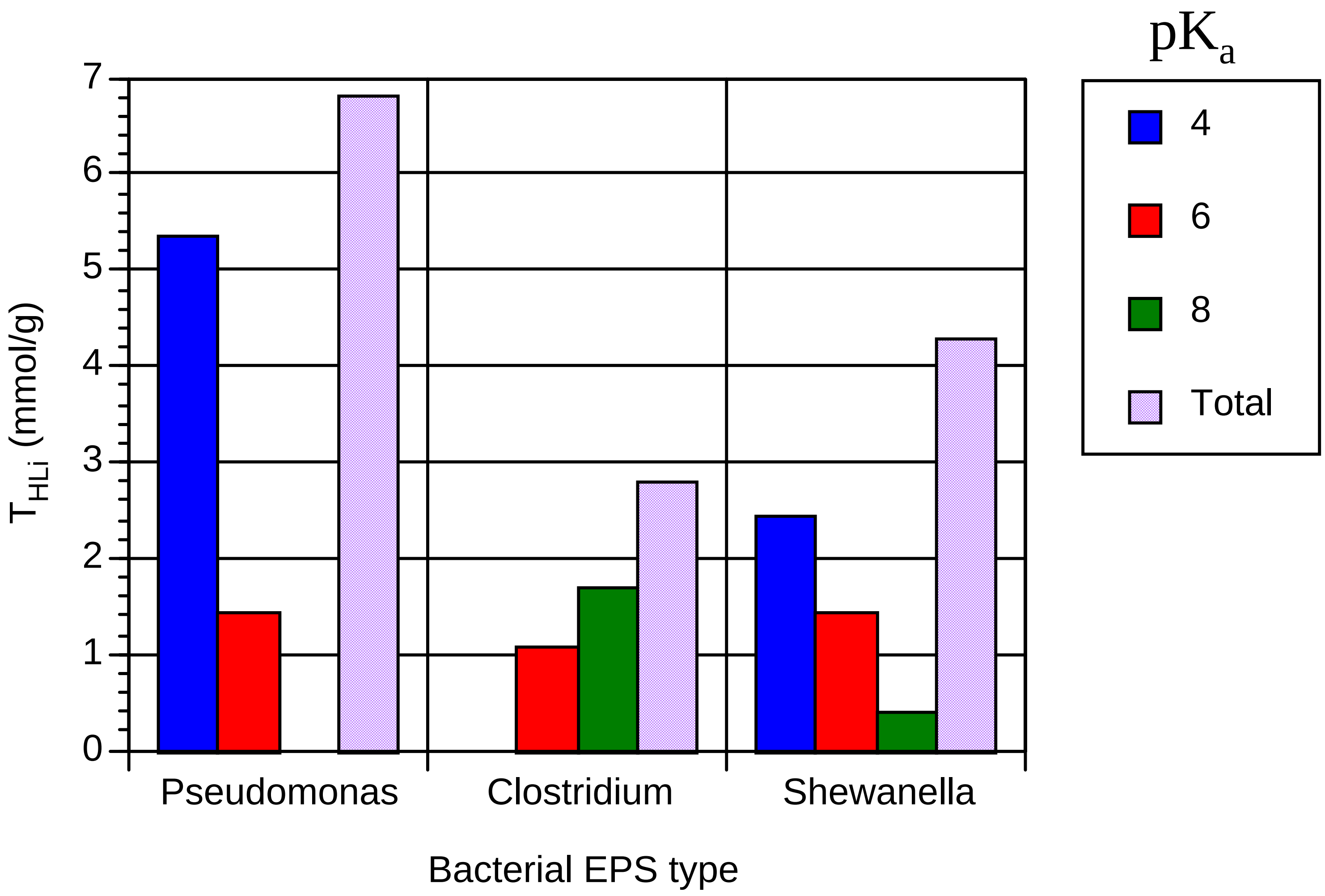


Affinity distributions

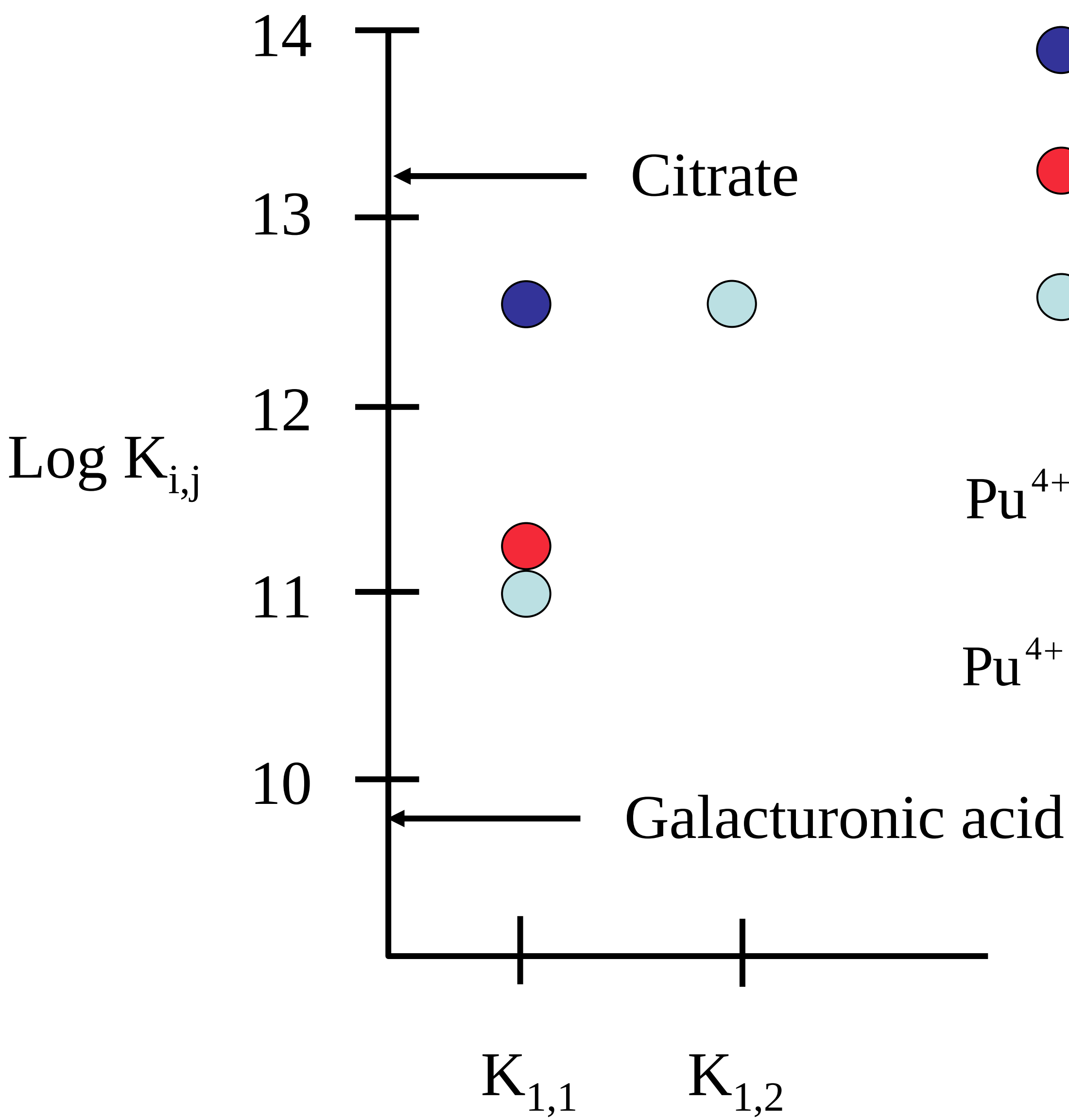
'Discrete' peaks



Comparison of EPS 'ligand' specific concentrations



Comparison of Pu / EPS binding



$$Pu^{4+} + HL_1 \overset{K_{1,1}}{=} PuL_1^{3+} + H^+$$

$$Pu^{4+} + 2HL_2 \overset{K_{1,2}}{=} Pu(L_2)_2^{2+} + 2H^+$$

Microbially-enhanced Pu mobilization

- Soil analysis
- Batch studies
- 'Static' columns

Radioactive Properties of Key Plutonium Isotopes

Isotope	Half-Life (years)	Specific Activity (Ci/g)	Decay Mode	Alpha (MeV)	Beta (MeV)	Gamma (MeV)
Pu-238	88	17	α	5.5	0.011	0.0018
Pu-239	24,000	0.063	α	5.1	0.0067	$<$
Pu-240	6,500	0.23	α	5.2	0.011	0.0017
Pu-241	14	100	β	$<$	0.0052	$<$
Pu-242	380,000	0.0040	α	4.9	0.0087	0.0014

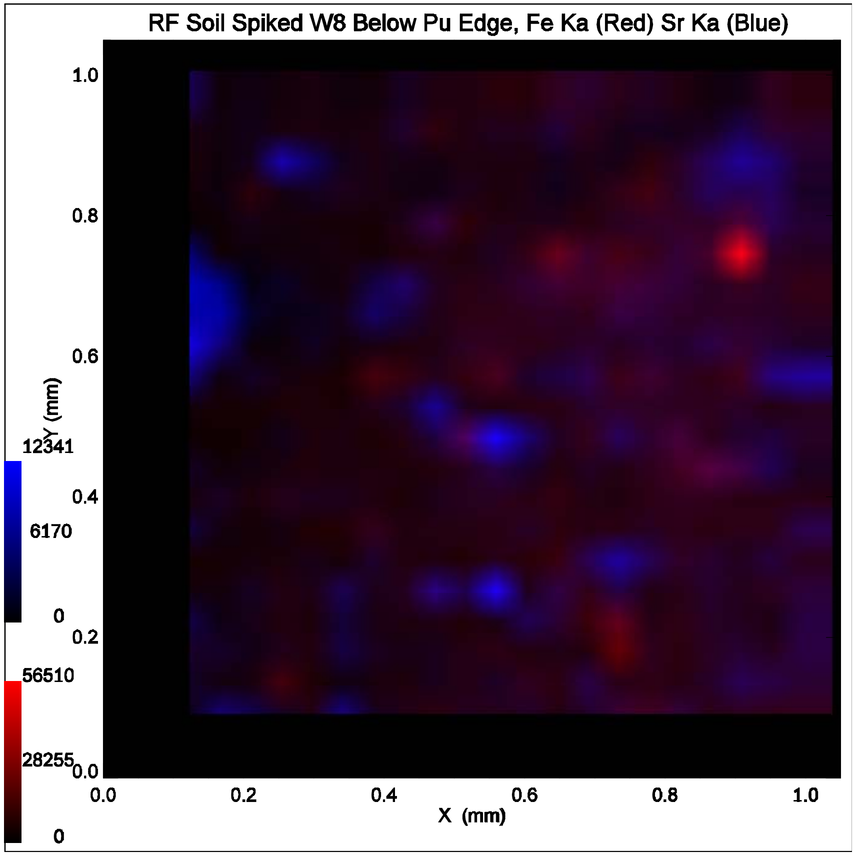
[US DOE, Plutonium Fact Sheet, ANL, 2001]

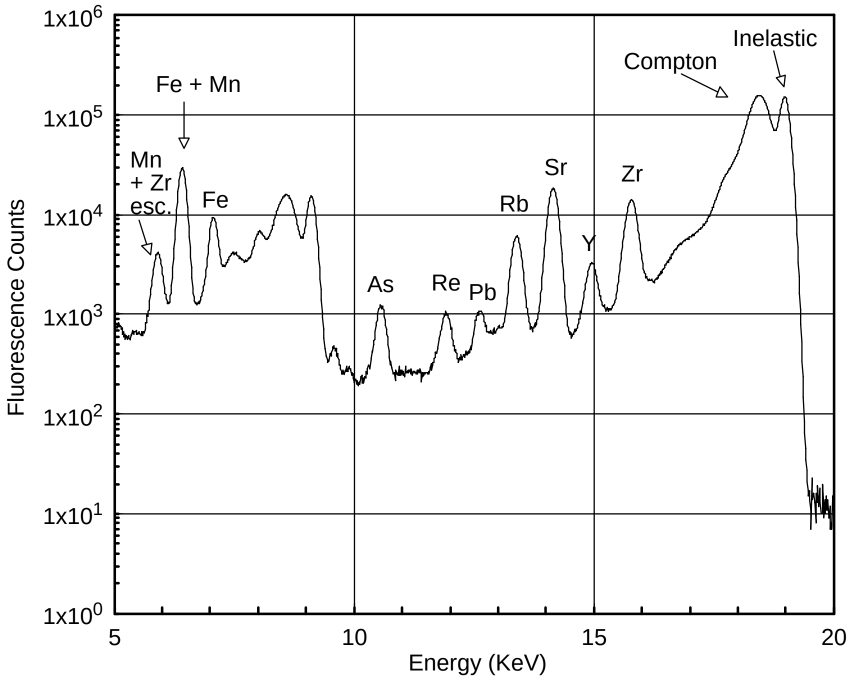
^{239,240}Pu: *in situ* ('aged)

^{241}Pu: tracer

^{242}Pu: tracer

μ-XRF of Rocky Flats Soil

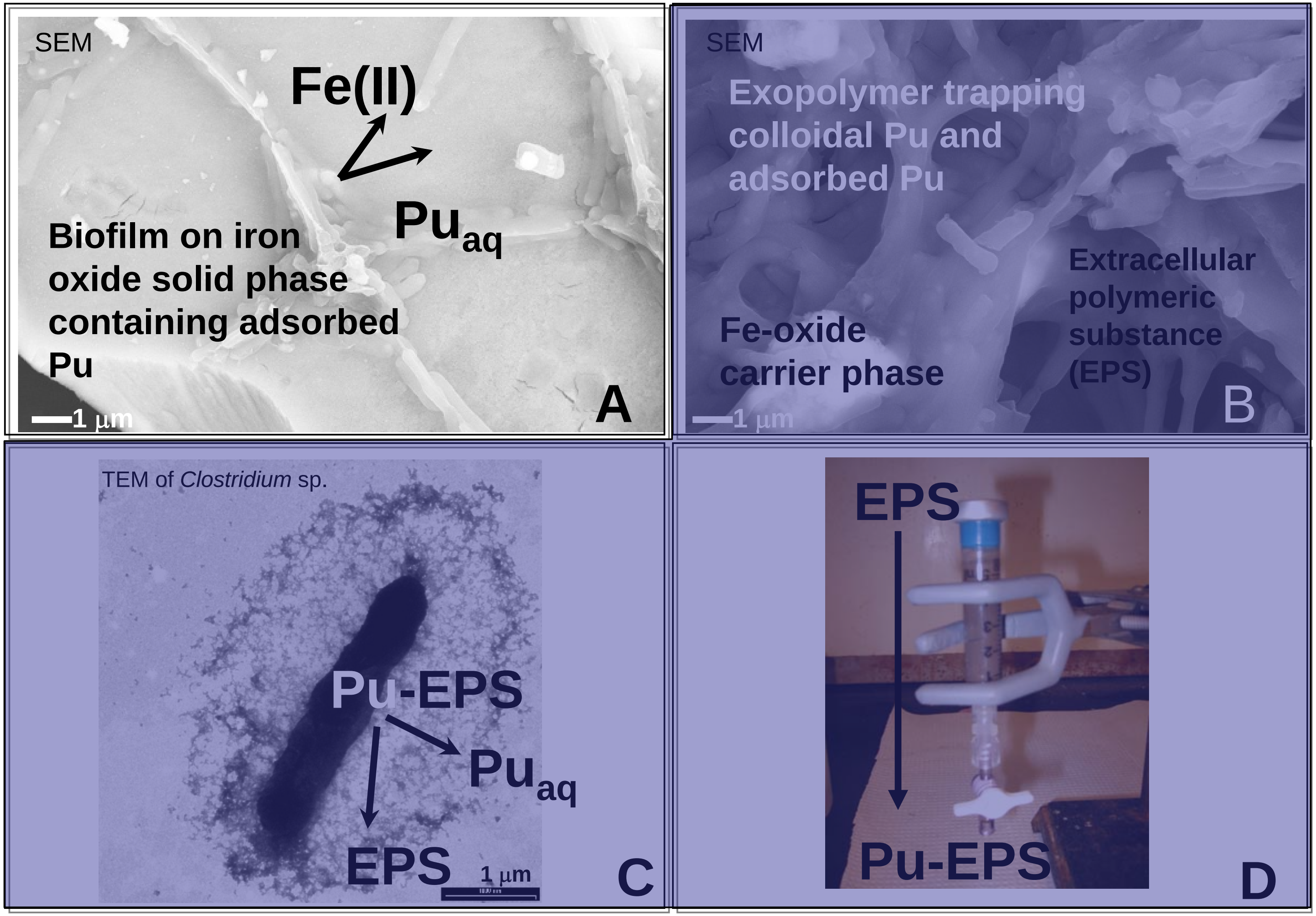




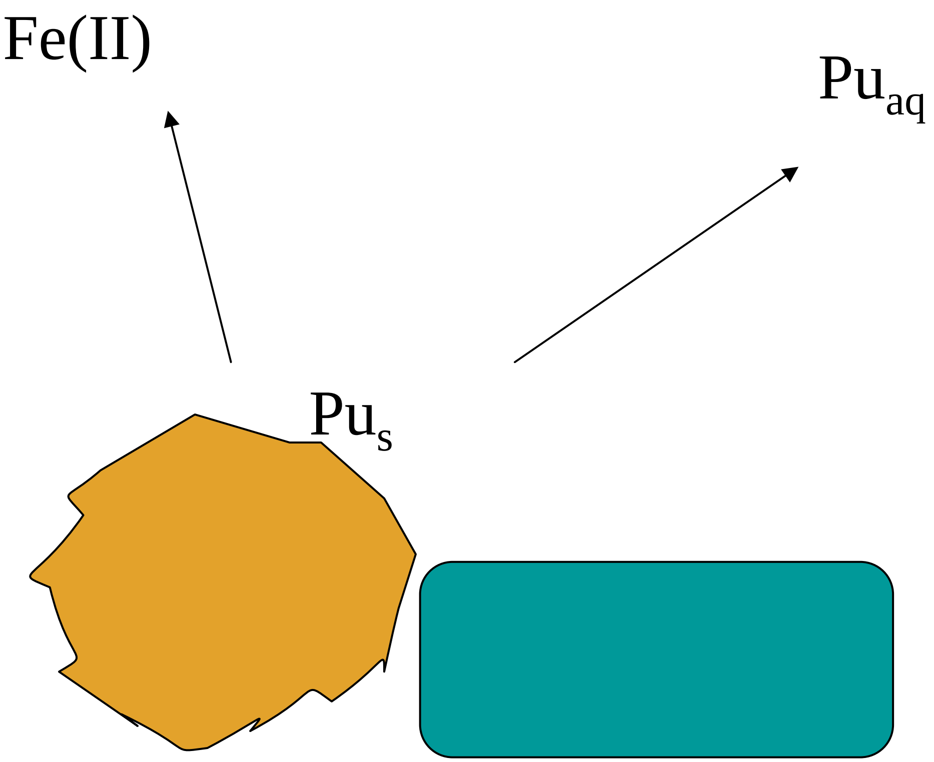
[1 mm² map of Fe (red) and Sr (blue) of 'as-rec'd' RF soil; incident beam energy 17.5 KeV]

[Microbeam (10 x 20 μm spot) X-ray fluorescence of RF soil elements characteristic of loam/sandy soil]

[Analyses performed at NSLS Envirosuite beamline X27A; note that 'as-rec'd' the soil contained 1.6 ng ^{239,240}Pu g^{-1} dry wt., 5 mg Fe g^{-1} dry wt. (0.5 wt. %), 1.97% TOC]

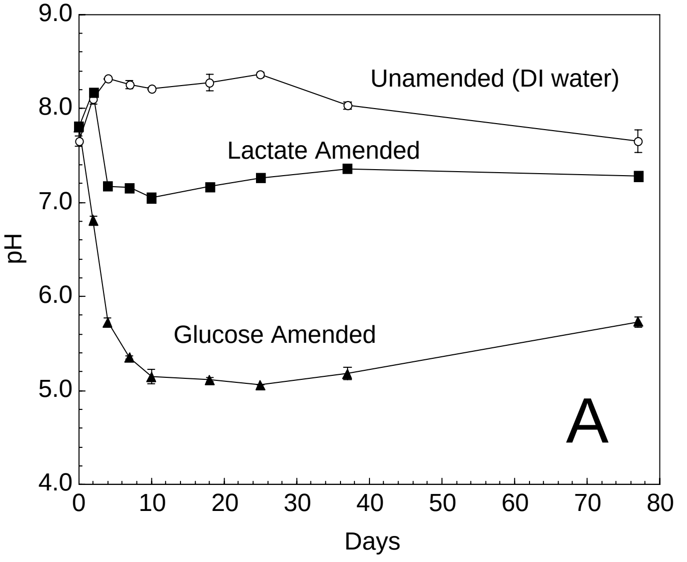


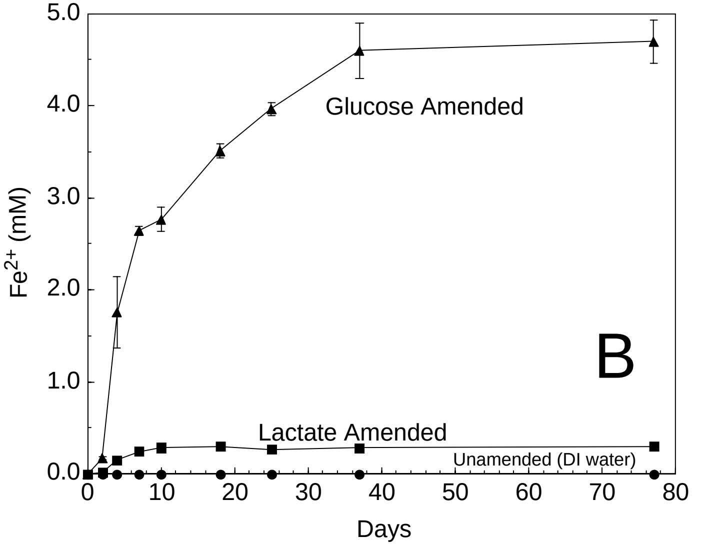
Key: **A**. Pu carrier-phase dissolution; **B**. Trapping of colloidal Pu by EPS; **C**. Biodegradation of Pu-EPS; **D**. Enhanced Transport of Pu by EPS.


Fe(II)
Pu_{aq}
Pu_s

Biotransformation of Pu in Soil from Rocky Flats

Incubation time = 10 days



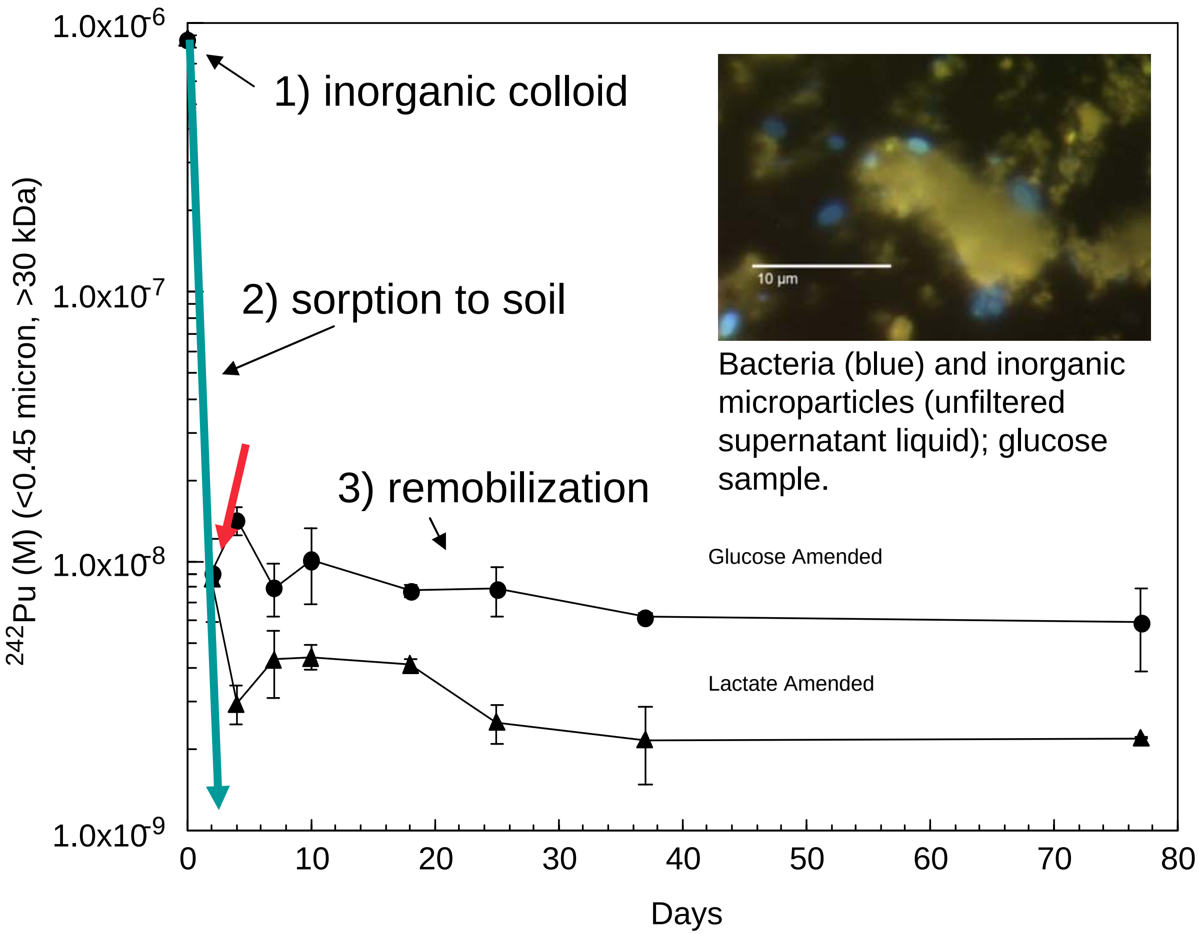


Biostimulation of RF soil:

A. The pH dropped to 7 due to metabolism of lactate; and to 5 due to glucose fermentation. 25 days, E_H glucose = -180 mV, lact. = -123 mV.

B. Glucose fermentation released 50 wt. % of the total reducible iron oxide from the soil; lactate metabolism released 3.5 wt. %.

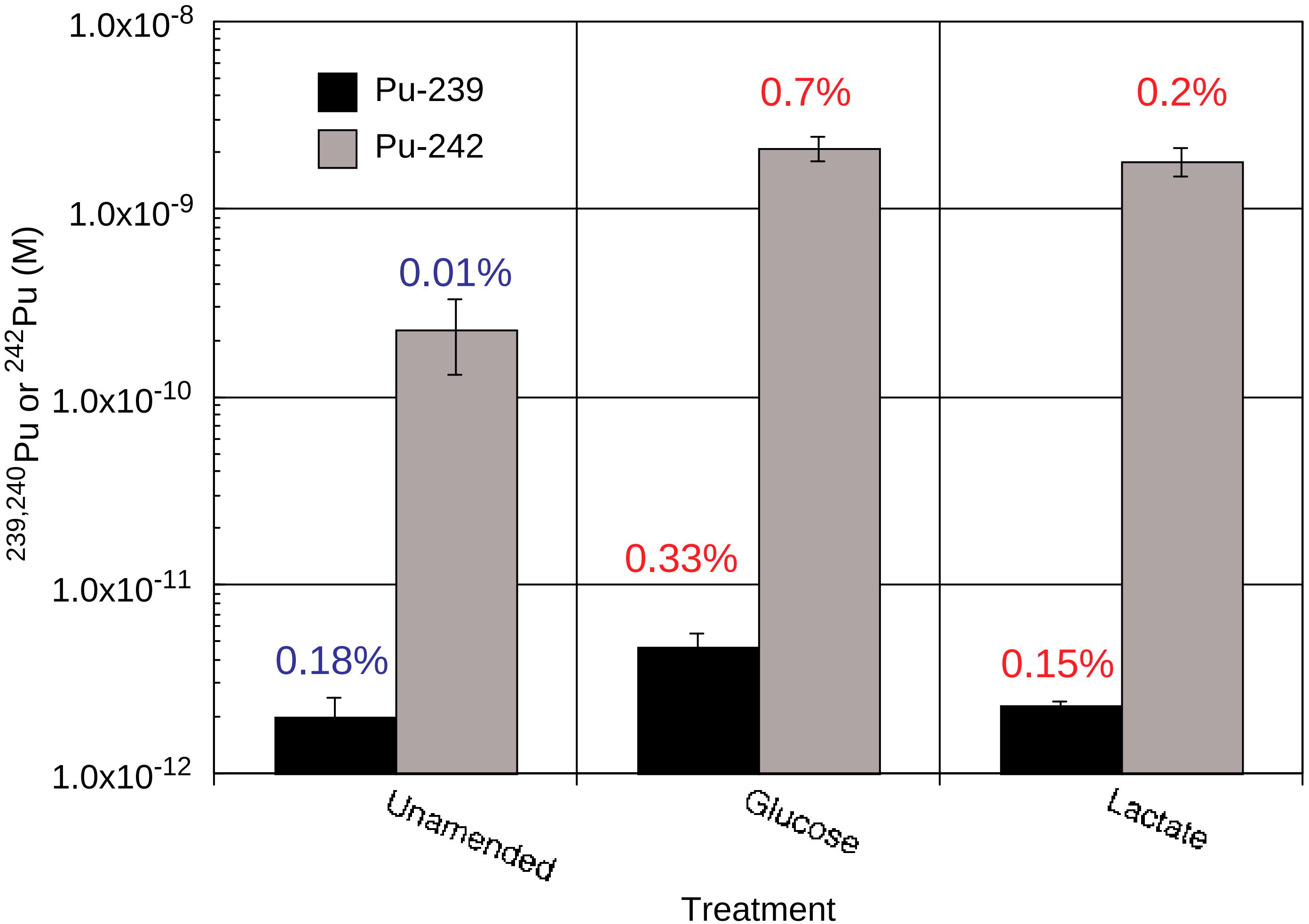
Formation of Pu Colloids in Soil due to Microbial Action



Bacteria (blue) and inorganic microparticles (unfiltered supernatant liquid); glucose sample.

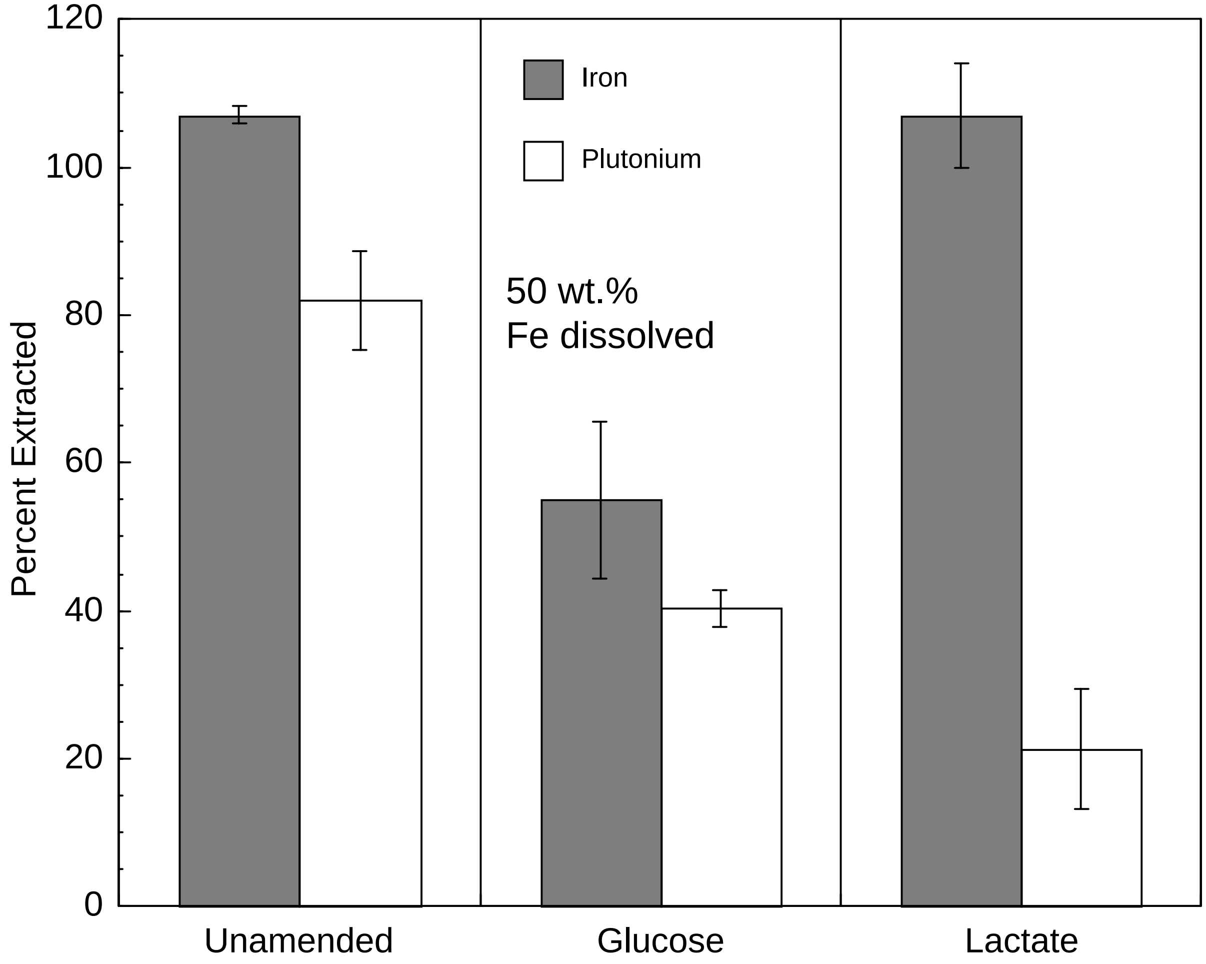
[^{242}Pu nitrate (1.5 x 10^{-6}M) added to soil resulted in 1) inorganic colloid formation, 2) sorption to soil and 3) remobilization as a colloid (<0.45 μm) upon incubation with glucose and lactate. <0.1% of total ^{242}Pu was in this fraction of unamended samples.]

$^{239,240}Pu$ and ^{242}Pu Fate at 77 Days Incubation



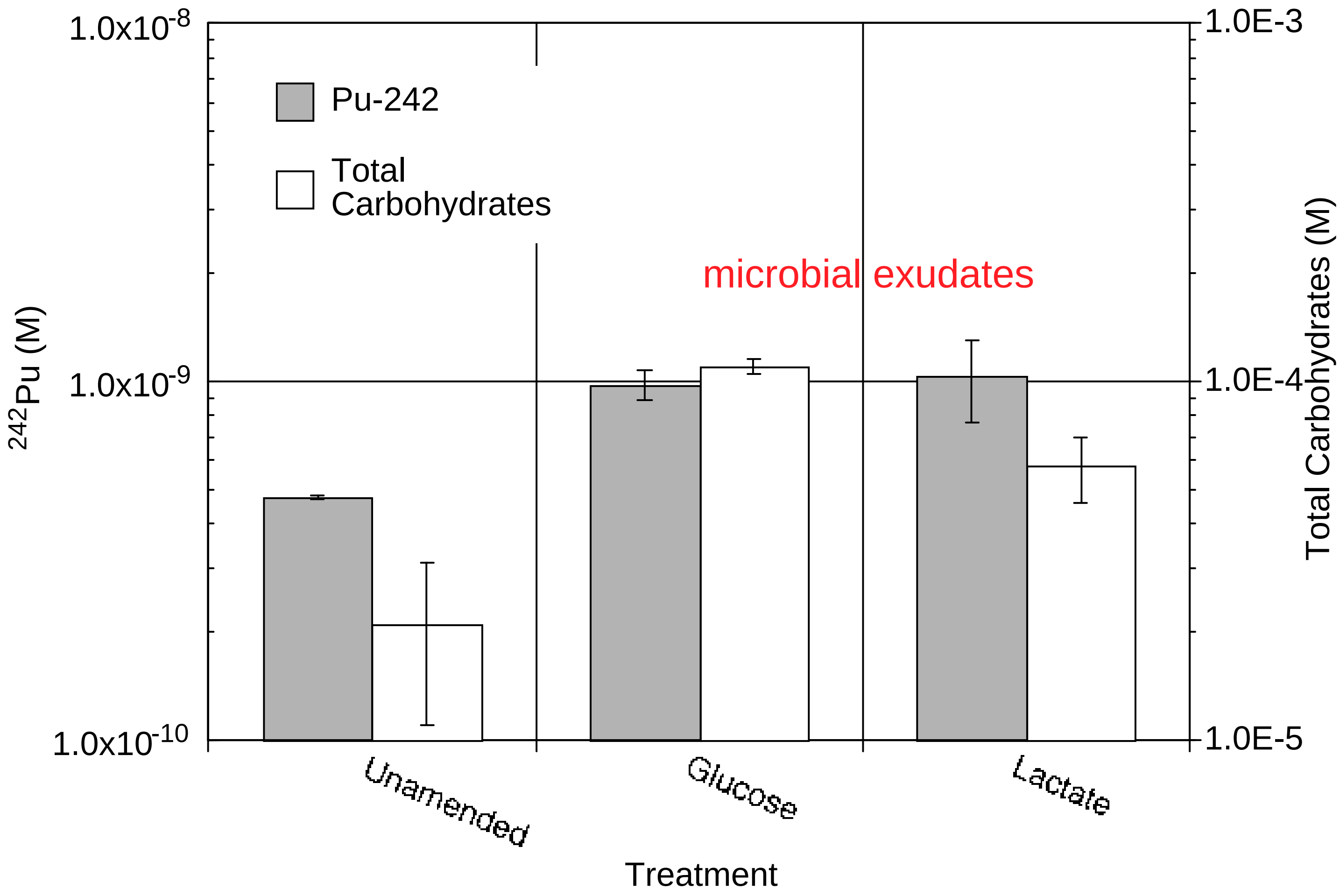
[Indigenous $^{239,240}Pu$ and spiked ^{242}Pu were mobilized (<0.45 μm) from RF soil with both glucose and lactate; the majority of the $^{239,240}Pu$ in the 'as-rec'd' soil was associate with the organic and inert fraction.] [mol % of total added]

Re-distribution of ^{242}Pu in Soil at 77 Days Incubation



[^{242}Pu was recovered with the reducible iron oxide fraction (CBD extraction) in unamended samples, however, it was redistributed to another phase after biostimulation with glucose or lactate]

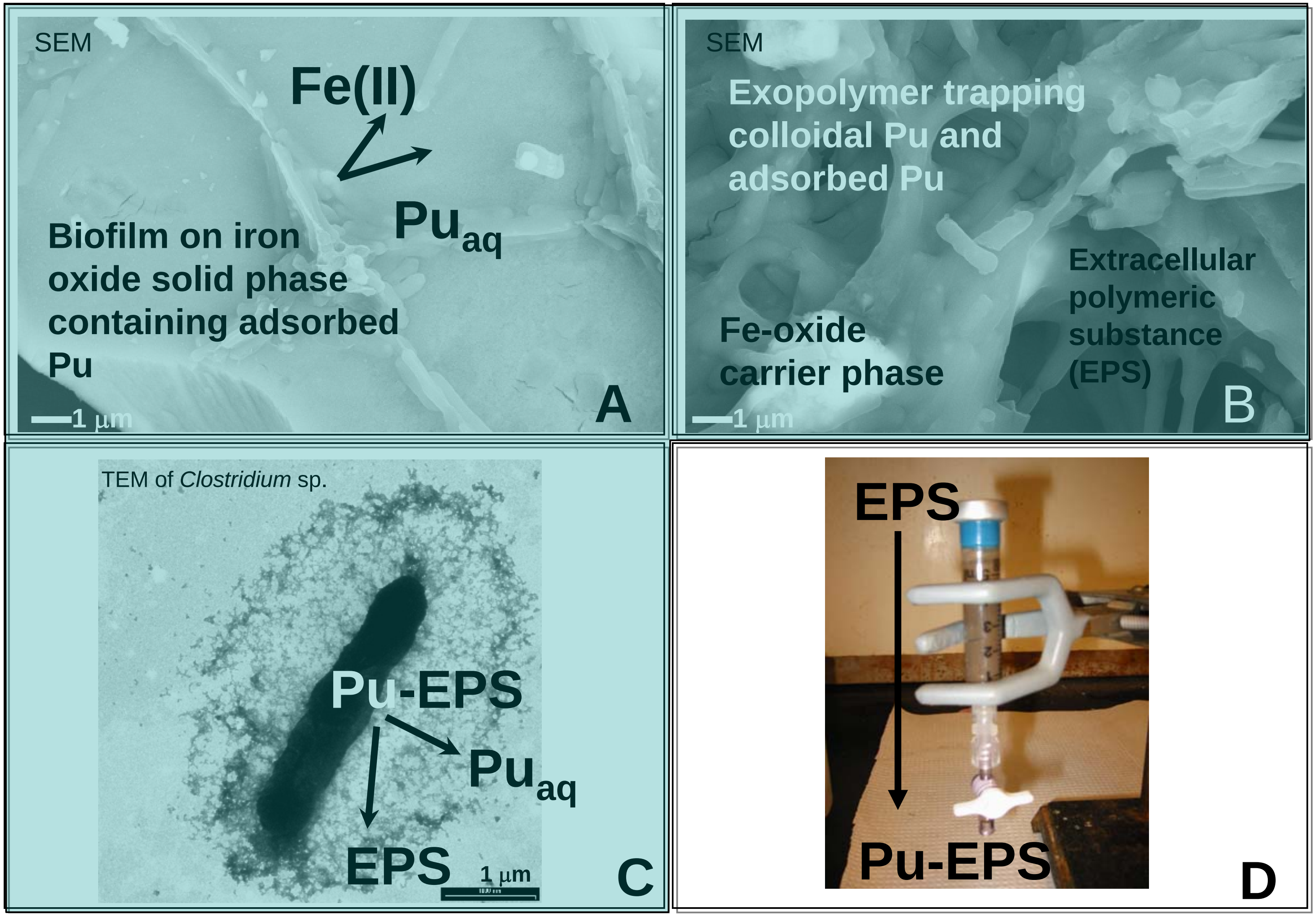
^{242}Pu and Total Carbohydrates



[At 77 days, the colloidal ^{242}Pu was correlated with an increase in suspended carbohydrates (<0.45 μm); this indicates that microbial exudates may play a role in Pu mobilization in the incubation experiments]

Summary of Soil Biotransformation Studies

- Pu was below detection for microprobe XRF, however discrete Fe phases were observed.
- Biostimulation with glucose released a significant amount of Fe while Fe(II) was readsorbed in lactate amended samples.
- A ^{242}Pu spike rapidly sorbed to soil, however it was remobilized due to microbial action; under highly reducing, fermentative conditions 0.7% of the total was detected in the <0.45μm, >30kDa fraction.
- The majority of Pu was released with the reducible iron oxide fraction of unamended samples, however this was not the case after biostimulation.
- The indigenous $^{239,240}Pu$ was remobilized, to a lesser extent than the spike, but this may be due to different mineralogical association (majority resided with the organic and inert fraction).
- There was an increase in soluble carbohydrates in the biostimulated samples implicating microbial exudates in stabilizing Pu in the colloidal fraction.

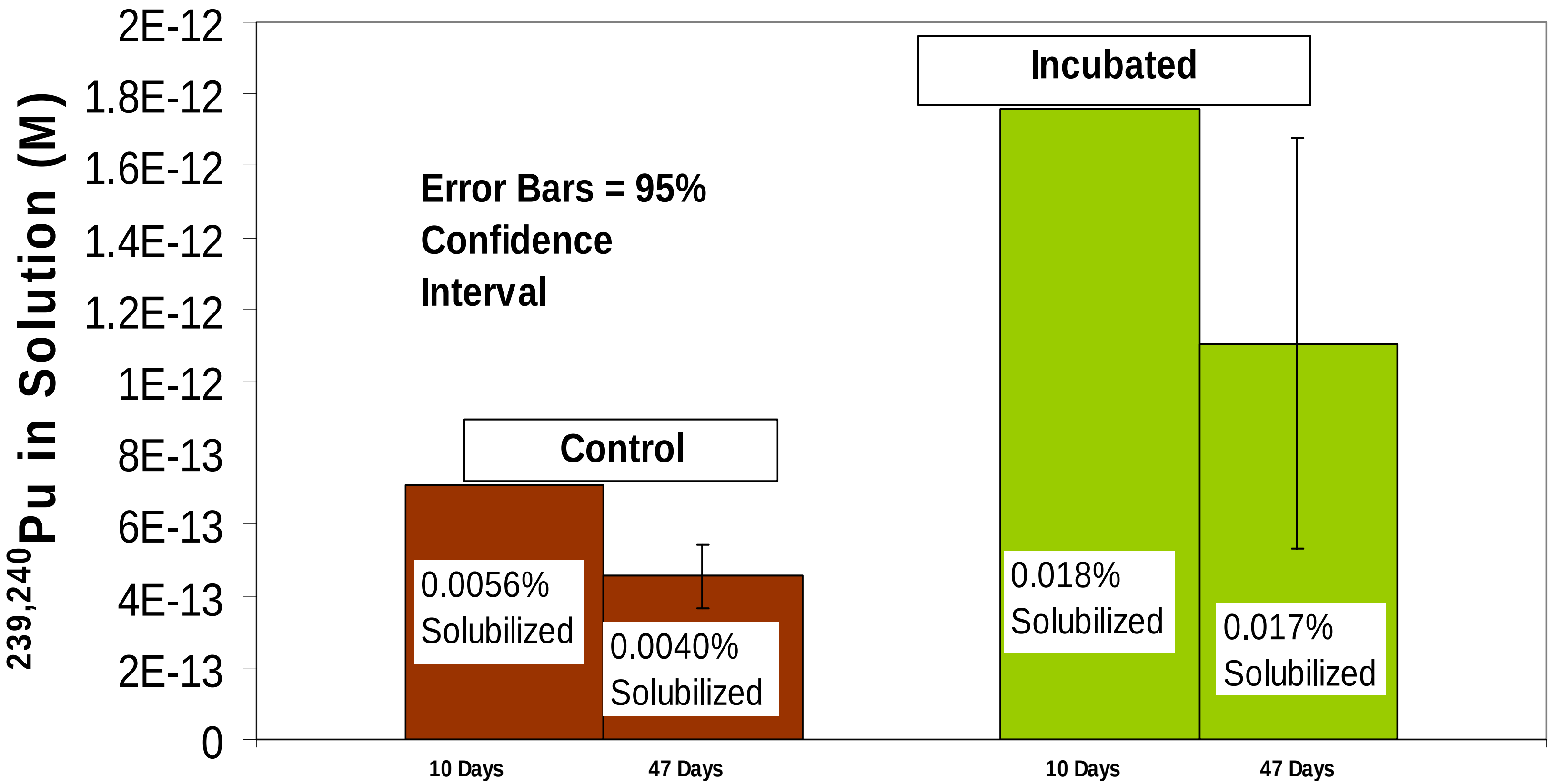


Key: **A**. Pu carrier-phase dissolution; **B**. Trapping of colloidal Pu by EPS; **C**. Biodegradation of Pu-EPS; **D**. Enhanced Transport of Pu by EPS.

'Static column' experiments

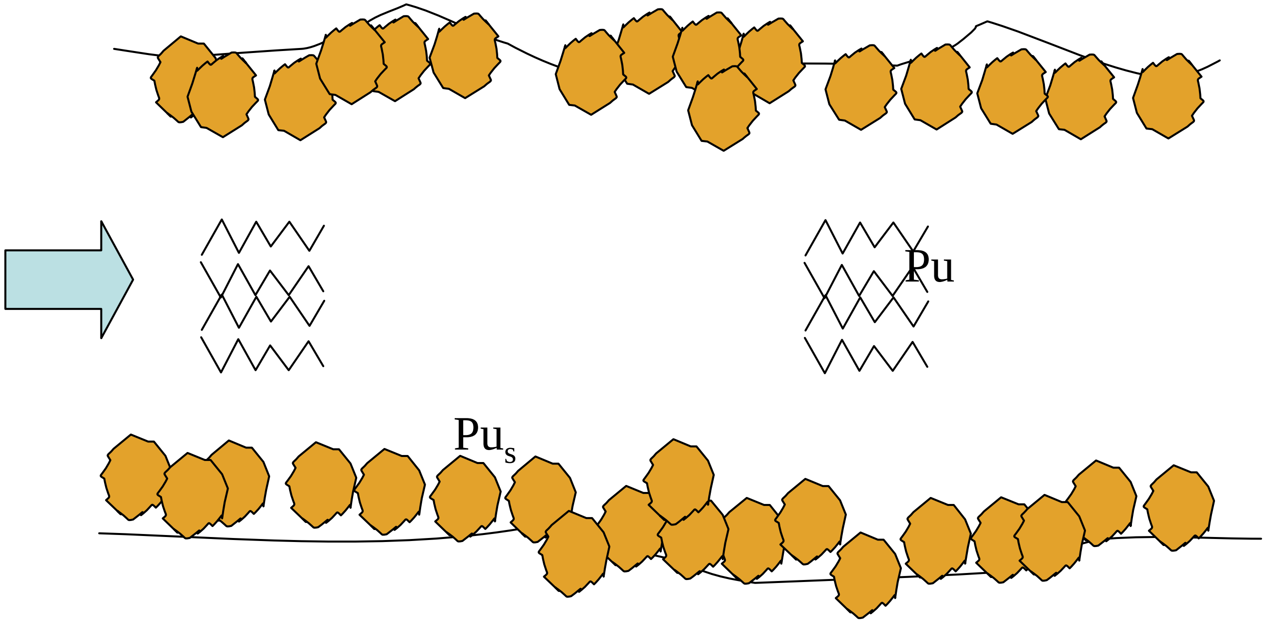
Assess Pu mobility under solid / solution ratios appropriate to *in situ* conditions

Static Column Incubation Experiment: 10 vs. 47 Day Incubation

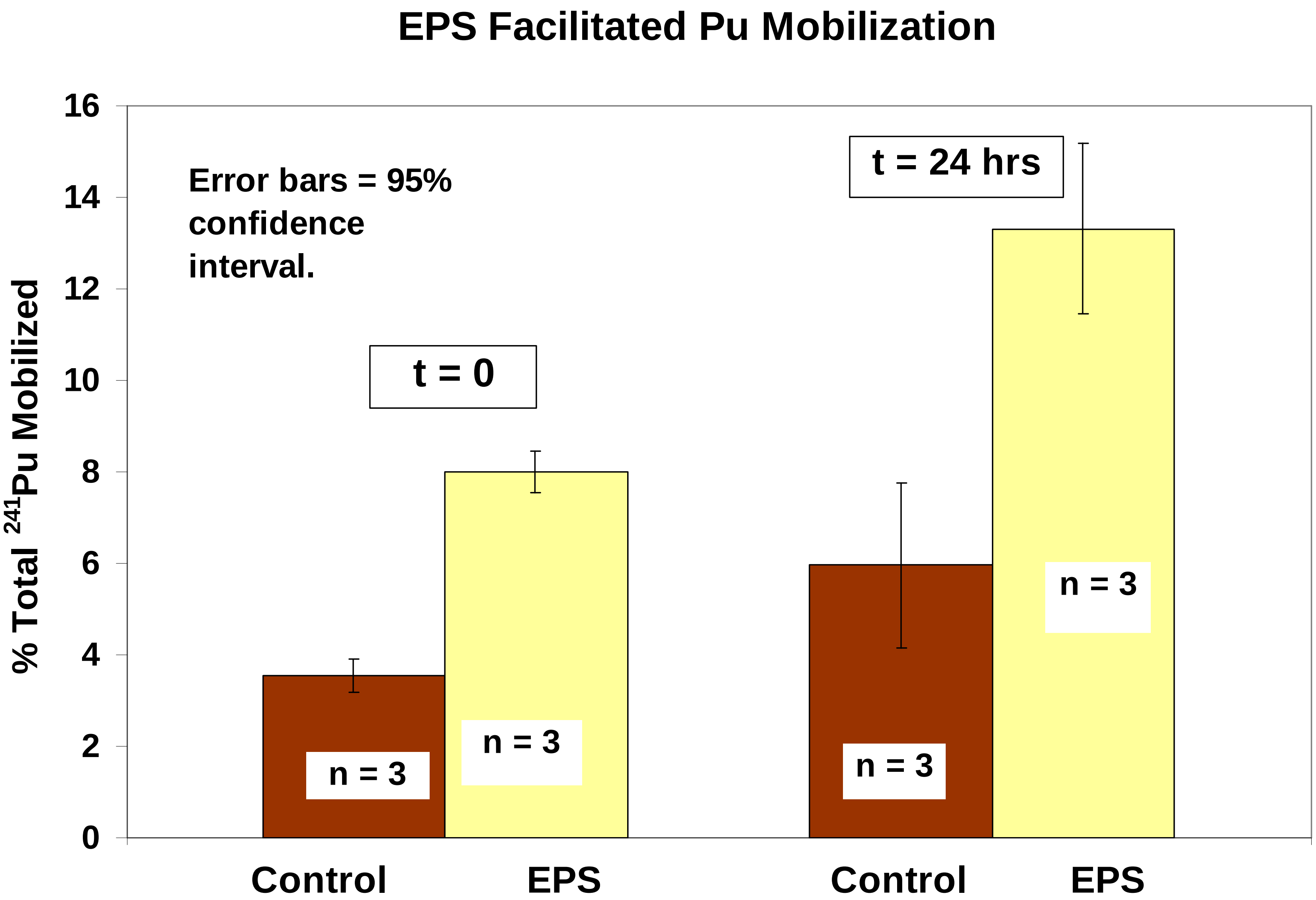


^{239,240}Pu = ~70 pCi / g; 0.5 w/v glucose; 0.015% w/v NH_4Cl

Pu transport enhancement by EPS

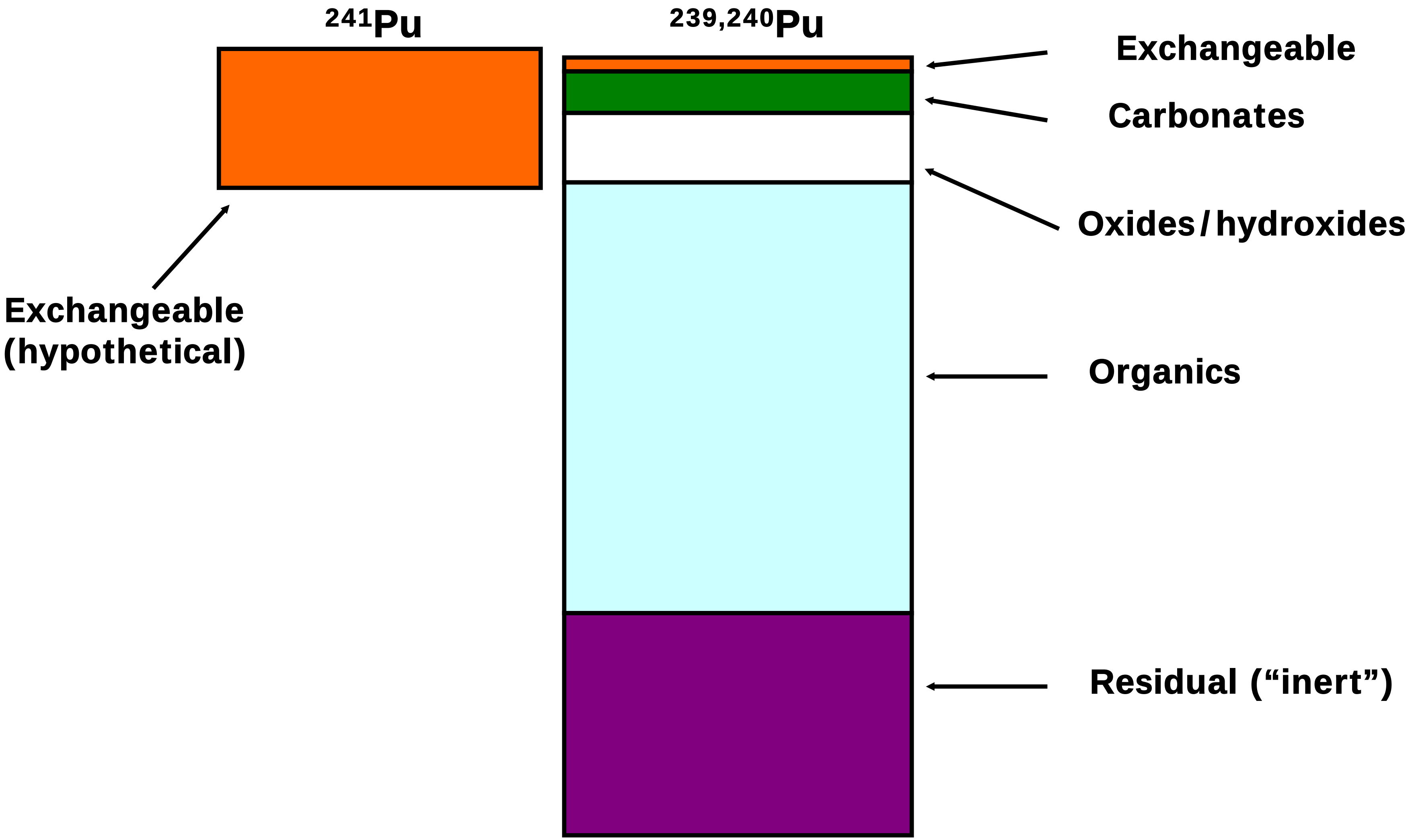


Sorbed Pu-241 to RF soil for 24hrs then 22 mg/L EPS (~ 10 mg/L OC) injected

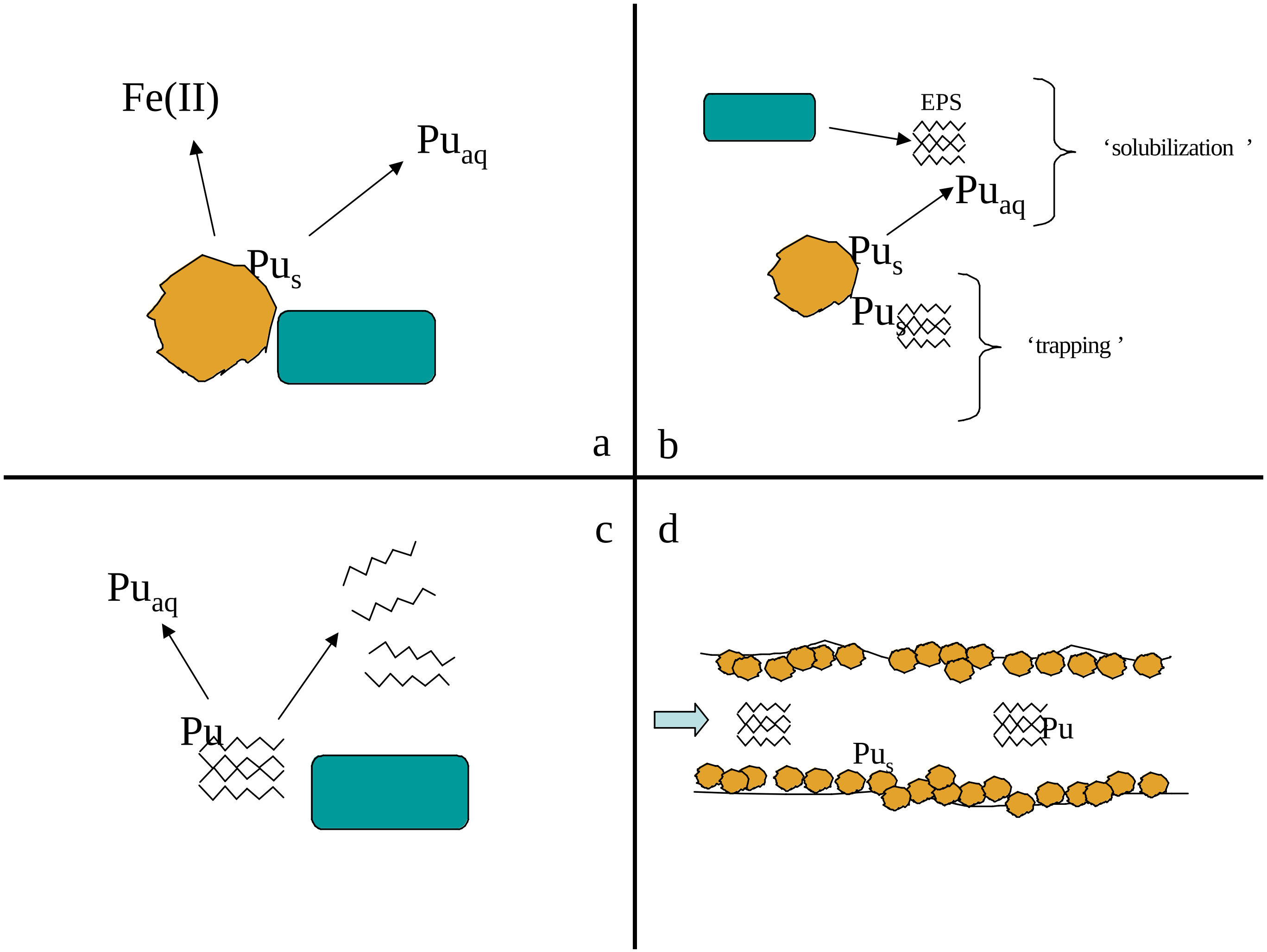


^{239,240}Pu mobilized: 0.018% v. 0.004% in the control

- <u>^{239,240}Pu vs. ^{241}Pu Tracer</u>



Summary:
Pu mobility as a transformational process



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- NABIR Program